AMERICAN FERN JOURNAL

Volume 75

1985

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Editor

Alan R. Smith
Department of Botany, University of California, Berkeley, CA 94720

Associate Editors

Gerald J. Gastony, Department of Biology, Indiana University,
Bloomington, IN 47401

Christopher Haufler, Department of Botany, University of Kansas,
Lawrence, KS 66045

David B. Lellinger, U. S. National Herbarium NHB-166, Smithsonian Institution,
Washington, D.C. 20560

Terry R. Webster, Biological Sciences Group, University of Connecticut,
Storrs, CT 06268

The American Fern Society

Council for 1985

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268. *President*
FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *Vice-President*
W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*
JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*
DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*
ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*
DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*
DENNIS Wm. STEVENSON, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns and fern allies. It is owned by the American Fern Society, published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405, and printed by Allen Press, Inc., 1041 New Hampshire St., Lawrence, KS 66044. Second-class postage paid at Burlington, VT, and additional entry point.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership $200.00).

Orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603. Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

Fiddlehead Forum

The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

Table of Contents

(A list of articles arranged alphabetically by author)

BARRINGTON, DAVID S., The present evolutionary and taxonomic status of the fern genus *Polystichum* 22
BOOM, BRIAN M., Ethnopteridology of the Chácobo Indians in Amazonian Bolivia 19
BRITTON, D. M. (see Kott) 44
BURKHALTER, JAMES R., A new station for *Dicranopteris flexuosa* in Bay County, Florida 79
BURNHAM, ROBYN J. (see Spicer et al.) 1
COX, PAUL ALAN AND P. B. TOMLINSON, Relationships between ecological pattern and branching in the tree fern *Lophosoria quadripinnata* in Veracruz, Mexico 105
DUCKETT, JEFFREY G., Wild gametophytes of *Equisetum sylvaticum* 120
ESTEVES, LUCIANO M., GIL M. FELIPPE, AND THEREZINHA S. MELHEM, Germination and morphology of spores of *Trichipteris corcovadensis* 92
FELIPPE, GIL M. (see Esteves et al.) 92
GLICKEN, HARRY (see Spicer et al.) 1
GRANT, PAUL (see Spicer et al.) 1
HASKINS, MELANIE L. (see Werth et al.) 128
HAUKE, RICHARD L., Ontogeny of the commissure of *Equisetum* 111
———, Gametophytes of *Equisetum giganteum* 132
HEPLER, PETER K. (see Wayne) 12
HOLDER, SAMUEL W. (see Landry) 133
HULBURT, AKKE (see Werth et al.) 128
IWATSUKI, KUNIO (see Kato) 73
JOHNSON, DAVID M., *Marsilea quadrifolia* and *M. vestita* in the floras of Kansas and Missouri 28
..... 30
———, New records for longevity of *Marsilea* sporocarps 30
KATO, MASAHIRO, and KUNIO IWATSUKI, An unusual submerged aquatic ecotype of *Asplenium unilaterale* 73
KOTT, L. S., and D. M. BRITTON, Role of morphological characteristics of leaves and the sporangial region in the taxonomy of *Isoetes* in northeastern North America 44
LANDRY, GARRIE P., and SAMUEL W. HOLDER, *Marsilea macropoda* new to Louisiana 133
LELLINGER, DAVID B., Nomenclatural notes on some ferns of Costa Rica, Panama, and Colombia.—II 31
———, review of *Arkansas ferns and fern allies* 37
———, review of *Ferns of Jamaica, a guide to the pteridophytes* 119
LUCANSKY, TERRY W., Anatomical studies of *Sphaeropteris* and *Cnemidaria* (Cyatheaceae) 80
MARSH, B. H. (see Toia et al.) 38
MCDONALD, J. W. (see Toia et al.) 38
MELHEM, THEREZINHA S. (see Esteves et al.) 92
MICKEL, JOHN T., Three new anemias from northern South America 33
——— (in Barrington) 24
MONTGOMERY, JAMES D., review of *The ferns and fern allies of Southern Africa* 31
ORZELL, STEVE L. (see Peck, James H., et al.) 71
PECK, CAROL J. (see Peck, James H., et al.) 71
PECK, JAMES H., STEVE L. ORZELL, ERIC SUNDELL, and CAROL J. PECK, *Dryopteris ludoviciana* and *D.* ×*australis* new to Arkansas 71
PERKINS, S. K. (see Toia et al.) 38
PETERS, G. A. (see Toia et al.) 38
PROCTOR, GEORGE R., New species of *Thelypteris* from Puerto Rico 56
RURY, PHILLIP M., New locations for *Isoëtes tegetiformans* in Georgia 102
SMITH, ALAN R., review of *Ferns to know and grow* 11
——— (in Barrington) 25
———, review of *Index filicum. Supplementum quintum: pro annis 1961–1975* 76

———, review of *Guide de fougéres et plantes alliées* 104
SMITH, DAVID K., *Polystichum aleuticum* from Adak Island, Alaska, a second locality for the species 72
SPICER, ROBERT A., ROBYN J. BURNHAM, PAUL GRANT, and HARRY GLICKEN, *Pityrogramma calomelanos*, the primary, post-eruption colonizer of Volcán Chichonal, Chiapas, Mexico 1
SUNDELL ERIC (see Peck, James H., et al.) 71
THOMAS, R. DALE, A newly discovered habitat for *Isoëtes melanopoda* in Louisiana 77
TOIA, R. E., JR., B. H. MARSH, S. K. PERKINS, J. W. MCDONALD, and G. A. PETERS, Sporopollenin content of the spore apparatus of *Azolla* 38
TOMLINSON, P. B. (see Cox) 105
WAGNER, DAVID H., JR. (in Barrington) 23
WAGNER, FLORENCE S., Bilateral spores in New World grammitid ferns 6
WAGNER, WARREN H., JR. (in Barrington) 22
WAYNE, RANDY, and PETER K. HEPLER, The atomic composition of *Onoclea sensibilis* spores 12
WERTH, CHARLES R., MELANIE L. HASKINS, and AKKE HULBURT, *Osmunda cinnamomea* forma *frondosa* at Mountain Lake, Virginia 128
WHITTIER, DEAN P., review of *Gametophytes of Ophioglossaceae* 18

Volume 75, Number 1, pages 1–32, issued 2 August 1985
Volume 75, Number 2, pages 33–72, issued 5 August 1985
Volume 75, Number 3, pages 73–104, issued 6 December 1985
Volume 75, Number 4, pages 105–136, issued 5 March 1986

AMERICAN FERN JOURNAL

Volume 76

1986

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Editor

Alan R. Smith
Department of Botany, University of California, Berkeley, CA 94720

Associate Editors

Gerald J. Gastony, Department of Biology, Indiana University,
Bloomington, IN 47401

Christopher Haufler, Department of Botany, University of Kansas,
Lawrence, KS 66045

David B. Lellinger, U. S. National Herbarium NHB-166, Smithsonian Institution,
Washington, D.C. 20560

Terry R. Webster, Biological Sciences Group, University of Connecticut,
Storrs, CT 06268

The American Fern Society

Council for 1986

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *President*

JUDITH E. SKOG, Biology Dept., George Mason University, Fairfax, VA 22030. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

JAMES D. MONTGOMERY, Ecology III, R.D. 1, Berwick, PA 18603. *Back Issues Curator*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Sciences, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

Fiddlehead Forum

The editors (Dennis Wm. and Jan Wassmer Stevenson) welcome contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

Table of Contents

(A list of articles arranged alphabetically by author)

ALVERSON, EDWARD R. and JOSEPH ARNETT, *Pellaea brachyptera* new to Washington 25
ARNETT, JOSEPH (see Alverson) 25
BARRINGTON, DAVID S., CATHY A. PARIS, and THOMAS A. RANKER, Systematic inferences from spore and stomate size in the ferns 149
BUSH, ELEANOR M., A second eastern North American occurrence for forked spleenwort, *Asplenium septentrionale* 26
CARTER, RICHARD and WAYNE R. FAIRCLOTH, *Osmunda cinnamomea* forma *frondosa* in the coastal plain of Georgia and Florida 189
CUSICK, ALLISON W., *Cystopteris tennesseensis* in West Virginia 99
FAIRCLOTH, WAYNE R. (see Carter) 189
HAUFLER, CHRISTOPHER H. (see Windham) 114
HICKEY, R. JAMES, *Isoëtes* megaspore surface morphology: Nomenclature, variation, and systematic importance 1
HICKOK, LESLIE G. (see Warne) 187
LLOYD, ROBERT M. (see Skog) 30
LUEBKE, NEIL T. (see Taylor) 21
MORAN, ROBBIN C., The neotropical fern genus *Olfersia* 161
MOYROUD, RICHARD (see Nauman) 55
NAUMAN, CLIFTON E., *Trichomanes* in Florida 179
——— and RICHARD MOYROUD, A new substrate for *Ophioglossum palmatum* in Florida 55
PARIS, CATHY A. (see Barrington et al.) 149
PARRIS, B. S., Corrections to *Index filicum* 47
PECK, JAMES H., Second locality for *Dryopteris carthusiana* in Arkansas 28
PRICE, M. G., review of *Polypodiaceous ferns of India* 190
RANKER, THOMAS A. and CHARLES R. WERTH, Active enzymes from herbarium specimens: Electrophoresis as an afterthought 102
———, (see Windham et al.) 141
———, (see Barrington et al.) 149
SKOG, JUDITH E. and ROBERT M. LLOYD, review of *Biology of pteridophytes* 30
SMITH, ALAN R., Revision of the neotropical fern genus *Cyclodium* 56
SOLTIS, DOUGLAS E. and PAMELA S. SOLTIS, Active enzymes from megaspores of *Marsilea* and *Regnellidium* 17
SOLTIS, PAMELA S. (see Soltis, D.) 17
TAYLOR W. CARL, review of *A field manual of the ferns & fern-allies of the United States and Canada* 29
———, (see Wagner, W. H., et al.) 129
TAYLOR, W. CARL and NEIL T. LUEBKE, Germinating spores and growing sporelings of aquatic *Isoëtes* 21
TRYON, ROLLA, Some new names and combinations in Pteridaceae 184
WAGNER, F. S. (see Wagner, W. H., et al.) 129
———, (see Wagner, W. H.) 33
WAGNER, W. H., Jr., F. S. WAGNER, and W. CARL TAYLOR, Detecting abortive spores in herbarium specimens of sterile hybrids 129
——— and ———, Three new species of moonworts (*Botrychium* subg. *Botrychium*) endemic in western North America 33
WALKER, GARY L. (see Warne) 187
WARNE, THOMAS R., GARY L. WALKER, and LESLIE G. HICKOK, A novel method for surface-sterilizing and sowing fern spores 187
WEBSTER, TERRY R. (see Whittier) 48
WERTH, CHARLES R. (see Ranker) 102

WHITTIER, DEAN P. and TERRY R. WEBSTER, Gametophytes of *Lycopodium lucidulum* from axenic culture 48
WINDHAM, MICHAEL D., The use of fern herbarium specimens in biosystematic research: Introduction 101
——— and CHRISTOPHER H. HAUFLER, Biosystematic uses of fern gametophytes derived from herbarium specimens 114
———, PAUL G. WOLF, and THOMAS A. RANKER, Factors affecting prolonged spore viability in herbarium collections of three species of *Pellaea* 141
WOLF, PAUL G. (see Windham et al.) 141

Volume 76, Number 1, pages 1–32, issued 28 May 1986
Volume 76, Number 2, pages 33–100, issued 21 August 1986
Volume 76, Number 3, pages 101–160, issued 23 October 1986
Volume 76, Number 4, pages 161–192, issued 14 January 1987

AMERICAN FERN JOURNAL

Volume 75

Number 1

January–March 1985

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

***Pityrogramma calomelanos*, the Primary, Post-eruption Colonizer of Volcán Chichonal, Chiapas, Mexico** — *Robert A. Spicer, Robyn J. Burnham, Paul Grant,* and *Harry Glicken* — 1

Bilateral Spores in New World Grammitid Ferns — *Florence S. Wagner* — 6

The Atomic Composition of *Onoclea sensibilis* Spores — *Randy Wayne* and *Peter K. Hepler* — 12

Ethnopteridology of the Chácobo Indians in Amazonian Bolivia — *Brian M. Boom* — 19

Special Report

The Present Evolutionary and Taxonomic Status of the Fern Genus *Polystichum* — David S. Barrington — 22

Shorter Notes

***Marsilea quadrifolia* and *M. vestita* in the Floras of Kansas and Missouri** — David M. Johnson — 28

New Records for Longevity of *Marsilea* Sporocarps — David M. Johnson — 30

Nomenclatural Notes on some Ferns of Costa Rica, Panama, and Colombia.—II — David B. Lellinger — 31

Reviews — 11, 18, 31

Information for Authors — Cover 3

MISSOURI BOTANICAL

AUG 0 P 1985

GARDEN LIBRARY

The American Fern Society

Council for 1985

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268. *President*

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *Vice-President*

MICHAEL I. COUSENS, Faculty of Biology, University of West Florida, Pensacola, FL 32504. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Department of Botany, University of Vermont, Burlington, VT 05401. *Records Treasurer*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH ..... Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER ..... Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER ..... U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER .... Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Jounal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VA 05401. Second-class postage paid at Burlington and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ichthyological Associates, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to Dr. David S. Barrington, Department of Botany, University of Vermont, Burlington, VT 05401.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $9.00 gross, $8.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $8.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $160.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

Fiddlehead Forum

Prof. Dennis Wm. Stevenson, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027, is editor of the newsletter "Fiddlehead Forum." The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcombed, and are tax-deductible. Inquiries should be address to the Secretary.

American Fern Journal 75(1):1–5 (1985)

Pityrogramma calomelanos, the Primary, Post-eruption Colonizer of Volcán Chichonal, Chiapas, Mexico

ROBERT A. SPICER
Life Sciences Department, Goldsmiths' College, Creek Road, London SE8 3BU, England
ROBYN J. BURNHAM
Department of Botany KB-15, University of Washington, Seattle, WA 98195
PAUL GRANT
Geology Department, Imperial College, London SW7 2BP, England
HARRY GLICKEN
U.S. Geological Survey, Cascades Volcano Observatory, 5400 MacArthur Blvd., Vancouver, WA 98661

The southern Mexican Volcán Chichonal, otherwise known as "El Chichón," (17°20′N, 93°12′W) erupted violently on 28 March 1982 at 2330 (local time), sending a column of ash and sulfur aerosol 16.8 km into the atmosphere. Further intermittent activity culminated in two major explosive eruptions on 3 April (1930 local time) and 4 April 1982 (0530 local time). During the final eruptions, column collapse gave rise to three major pyroclastic surges and flows that devastated 154 km^2 of paratropical rain forest (*sensu* Wolfe, 1979) and agricultural land (Fig. 1). More complete descriptions of the eruptions are to be found in Varekamp et al. (1982), SEAN Bulletin (1982), Duffield et al. (1984) and Sigurdsson et al. (1985).

The pre-eruption vegetation of the area consisted of a patchwork of second-growth rain forest and local crops, which included cocoa, bananas, coffee, and maize. Numerous epiphytic pteridophytes were to be found in the area, with *Selaginella* aff. *schizobasis* Bak. (*Burnham 001*, BM, MEXU), *Blechnum occidentale* L. (*Burnham 09*, BM, MEXU), and *Pteris grandifolia* L. (*Burnham 065*, BM, MEXU) as frequent components of the ground cover.

The eruptions produced no lava flows, but trachyandesitic air-fall ash and pyroclastic surges and flows inundated a roughly circular area within a radius of approximately 6 km from the crater. The nature of the volcaniclastic deposits is described in detail in Sigurdsson et al. (1985). A particular feature of the eruptions was the large amount of sulfur ejected into the atmosphere (Thomas, 1982; Mroz & Sedlacek, 1982; Rampino & Self, 1984) and in the tephra (Varekamp & Luhr, 1982; Luhr et al., 1982). Chemical analyses of the tephra are given in Hoffer et al. (1982) and Cochemé and Demant (1983).

High local annual precipitation (>200 cm) (Rzedowski, 1983) subsequently produced numerous erosional gulleys that have cut down, in some cases through several meters of volcanic debris, to the 1982 pre-eruption soil horizon.

In February 1984 we spent four weeks in the Chichonal area. The purpose of our visit was to study the formation of potential plant fossil deposits associated

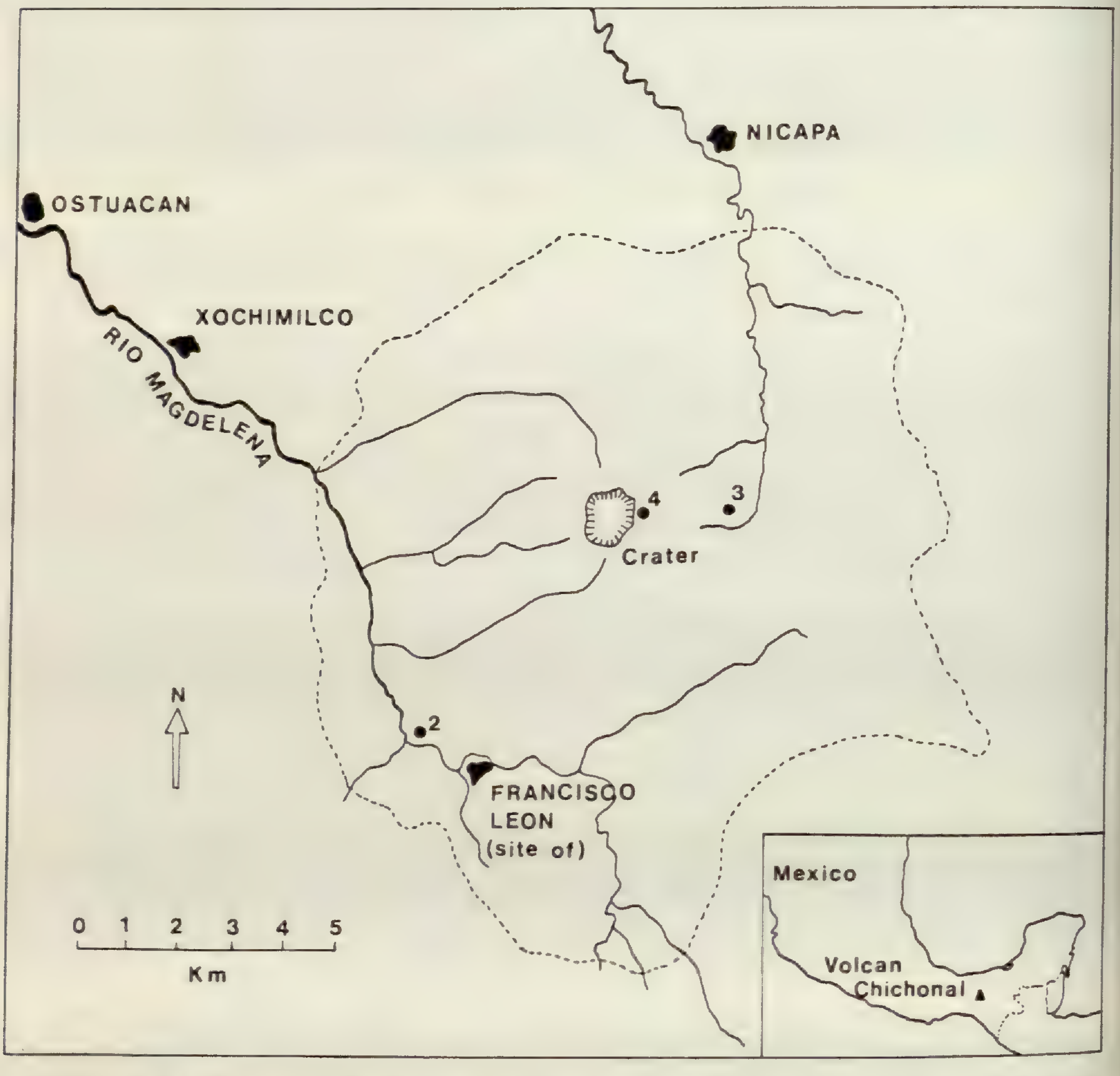
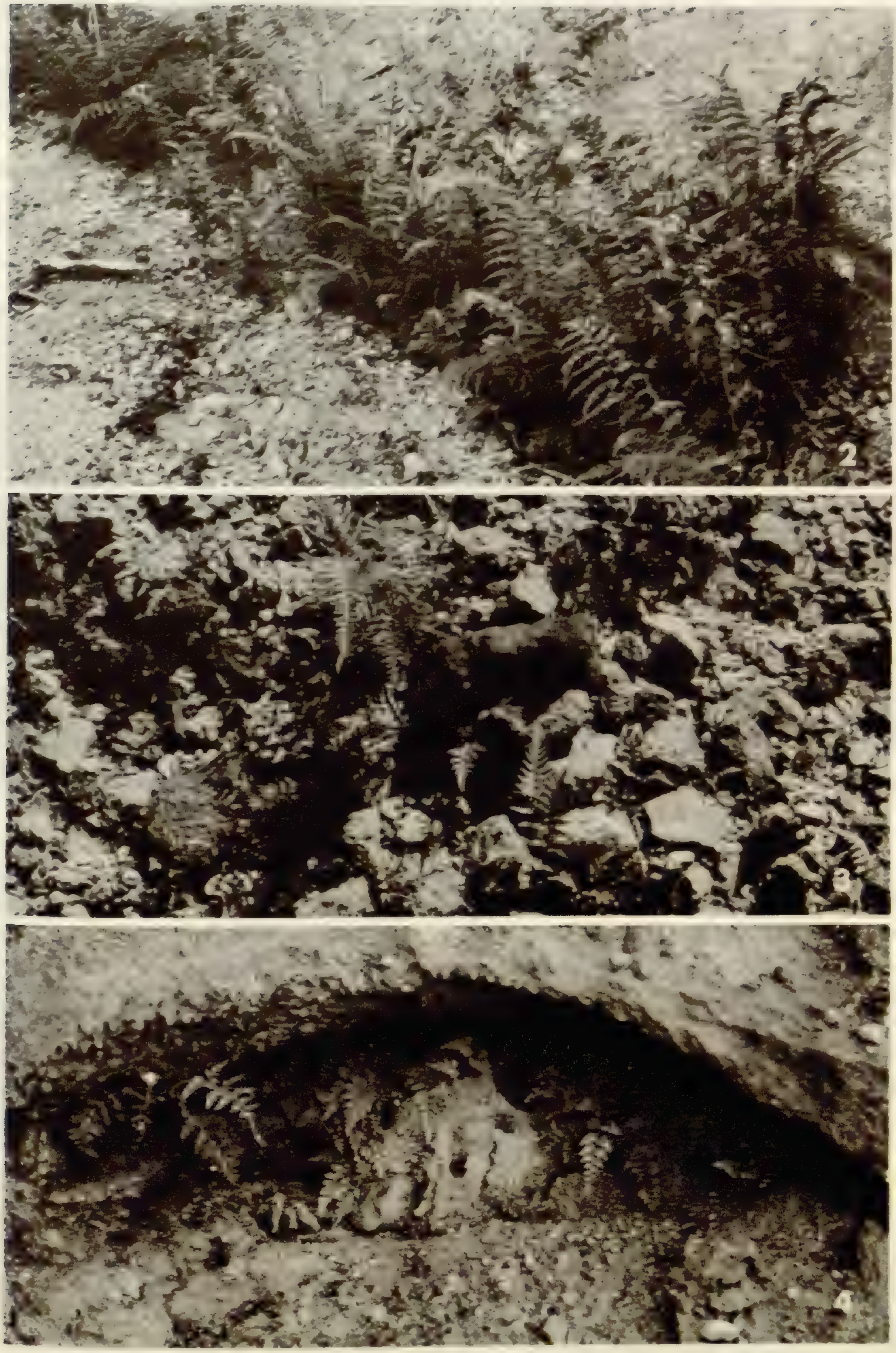


FIG. 1. Map of the Volcán Chichonal area. The dotted line indicates the approximate extent of the devastated area. Sites of Figs. 2–4 are indicated.

with the eruptions. This work involved studying plant and volcanic sediment distribution in the area, and it was immediately apparent that *Pityrogramma calomelanos* (L.) Link (*Burnham 055, 058*, BM, MEXU) was the primary colonizer wherever devastation of the pre-eruption vegetation had been intense. By February 1984, fertile fronds of *P. calomelanos*, up to 1 m long, were observed to have regenerated from rhizomes in the 1982 pre-eruption soil horizon. Furthermore, this regrowth had apparently stabilized the soil against further erosion (Fig. 2). Regrowth from buried rhizomes accounted for the largest plants of *Pityrogramma* that were observed, but these only represented a small proportion of the overall population in the devastated area. By far the greatest number of individuals occurred as young sporophytes, typically 5–7 cm high, at densities of approximately 40 per m^2 on stable, pumice-strewn slopes to within 1–2 km of the crater (Fig. 3). Young sporophytes were usually rooted in tephra the size of



FIGS. 2–4. *Pityrogramma calomelanos*. 2. Plants regenerating from buried rhizomes in erosional gullies 5.3 km from crater. 3. Young sporophytes on pumice-strewn slopes 2 km from crater. 4. Plants in crevasses 200 m from crater rim.

fine to medium sand between pumice blocks averaging 5–10 cm in maximum diameter. With decreasing distance from the crater, the sporophyte density decreased and became more patchy. However, sporophytes with fronds of 15 cm or so long were observed in small rock crevasses approximately 200 m east of the new crater rim (Fig. 4). Other notable occurrences were at the edge of eroded sulfur crusts surrounding non-active fumarolic sites on the distal lobes of large pyroclastic flows and on post-eruption flood deposits in the Río Magdelena river valley downstream from Ostuacán.

Pityrogramma is a genus native to tropical America. It is common in open habitats and is a colonizer of disturbed ground. Consequently it frequently occurs as a weed in palm and banana plantations (Tryon & Tryon, 1982). Although some authors consider the genus to be native to Africa and Madagascar (Tryon & Tryon, 1982), others consider it as introduced in the Old World, from where it has spread rapidly throughout the wet tropics (Schelpe, 1975). Its natural habitats include gravel bars in streams and rivers, rocky cliffs, and landslide areas, and it was noted as an early colonizer of laval flows on Mount Lamington, Papua (Schelpe, 1975). Schelpe (1975) stated that *Pityrogramma* was recorded on Krakatoa in 1906; he suggested that its arrival there was due to long-distance dispersal of spores by wind.

Because of the high sulfur content of the plume from Volcán Chichonal it is evident that *P. calomelanos* is tolerant of high sulfur concentrations in the substrate. Buried rhizomes were undoubtedly exposed to leached sulfurous compounds from the overlying volcaniclastics, and both prothalli and young sporophytes have been able to survive high local concentrations of sulfur in close proximity to fumaroles. In February 1984, sulfurous gases were still seeping from some pyroclastic flows, and large quantities of hydrogen sulfide were being given off from the crater. It would seem, therefore, that *Pityrogramma* is also tolerant of high concentrations of atmospheric sulfur and acidic rain.

Seed plants are recolonizing the devastated area, but none so prolifically as *Pityrogramma*. It is easy to see why this is so. The rhizomes are capable of surviving violent burial and some degree of thermal shock, and are able to produce fertile fronds relatively quickly. As with most homosporous pteridophytes, vast numbers of wind-dispersed spores are produced, any one of which has the capacity to produce a prothallus. Even if prothalli are spatially separated so as to prevent outcrossing, self-fertilization can give rise to a new sporophyte in a relatively short time. On the other hand, seed plants in the same situation may take longer to mature, flower, and set seed. Seeds are not produced in such large numbers as spores and are usually not dispersed so far.

High relative concentrations of monolete fern spores have recently been reported from coals immediately overlying an iridium-rich clay at the Cretaceous/Tertiary boundary in New Mexico and Colorado (Pillmore et al., 1984). This fern "spike" is interpreted as representing the initial floral recovery phase following a possible terminal Cretaceous bolide impact as suggested by Alvarez et al. (1980). The present environment surrounding El Chichón is in some ways analogous to that hypothesized for the immediate post-impact terrestrial Tertiary ecosystem.

We thank the following for their kind assistance: Servando de la Cruz, UNAM;

Mario Sousa and the staff of the Herbário Nacional de México; Sr. Isidro Chavez; the people of Ostuacán; and The Royal Society.

Literature Cited

Alvarez, L. W., W. Alvarez, F. Asaro, and H. V. Michel. 1980. Extraterrestrial cause for the Cretaceous-Tertiary extinction. Science 208:1095.

Cochemé, J. J. and A. Demant. 1983. Naturaleza y composición del material emitido por el Volcán Chichonal, Chiapas (marzo–abril, 1982). In *El Volcán Chichonal*. Mexico, D.F.: Universidad Nacional Autónoma de México.

Duffield, W. A., R. I. Tilling, and R. Canul. 1984. Geology of El Chichón volcano, Chiapas, Mexico. J. Volc. Geotherm. Res. 20:117–132.

Hoffer, J., G. P. Filiberto, and P. Muela. 1982. Eruption of El Chichón volcano, Chiapas, Mexico, 28 March to 7 April 1982. Science 218:1307–1308.

Luhr, J., I. S. E. Carmichael, and J. C. Varekamp. 1982. Minerology and petrology of ejecta from the March–April 1982 eruptions of El Chichón volcano, Chiapas, Mexico. Eos. Trans. Amer. Geophys. Union 63:1126–1127.

Mroz, E. J. and W. A. Sedlacek. 1982. Stratospheric aerosols from El Chichón. Eos. Trans. Amer. Geophys. Union 63:900.

Pillmore, C. L., R. H. Tschudy, C. J. Orth, J. S. Gilmore, and J. D. Knight. 1984. Geologic framework of nonmarine Cretaceous–Tertiary boundary sites, Raton Basin, New Mexico and Colorado. Science 223:1180–1183.

Rampino, M. R. and S. Self. 1984. The atmospheric effects of El Chichón. Sci. Amer. 250:34–43.

Rzedowski, J. 1983. *Vegetación de México*. Mexico, D.F.: Editorial Limusa.

Schelpe, E.A.C.L.E. 1975. Observations on the spread of the American fern *Pityrogramma calomelanos*. Fern Gaz. 1:101–104.

SEAN Bulletin, Smithsonian Institution. 1982. Volcanic events: El Chichón. 7:2–6.

Sirgurdsson, H., S. N. Carey, and J. M. Espindola. 1985. The 1982 eruptions of El Chichón volcano, Mexico: Stratigraphy of pyroclastic deposits. J. Volc. Geotherm. Res. (in press).

Thomas, G. E. 1982. Satellite observations of the El Chichón volcanic aerosol in the stratosphere. Eos. Trans. Amer. Geophys. Union 63:898.

Tryon, R. M. and A. F. Tryon. 1982. *Ferns and allied plants with special reference to tropical America*. New York: Springer-Verlag.

Varekamp, J. C. and J. Luhr. 1982. The 1982 eruption of El Chichón volcano: Nature of the eruption, ash and volatiles. Eos. Trans. Amer. Geophys. Union 63:1126.

———, ———, K. Prestegaard, and J. Canepa. 1982. The 1982 eruption of El Chichón volcano, Chiapas, Mexico. Part 1. Stratigraphy, volume and volatile element characteristics of the ash fall deposits. Geol. Soc. Amer. Abstr. Prog. 14:637.

Wolfe, J. A. 1979. Temperature parameters of humid to mesic forests of eastern Asia and relation to forests of other regions of the northern hemisphere and Australasia. U.S. Geol. Surv. Prof. Paper 1106:1–37.

American Fern Journal 75(1):6–11 (1985)

Bilateral Spores in New World Grammitid Ferns

FLORENCE S. WAGNER
Department of Botany, University of Michigan, Ann Arbor, MI 48109

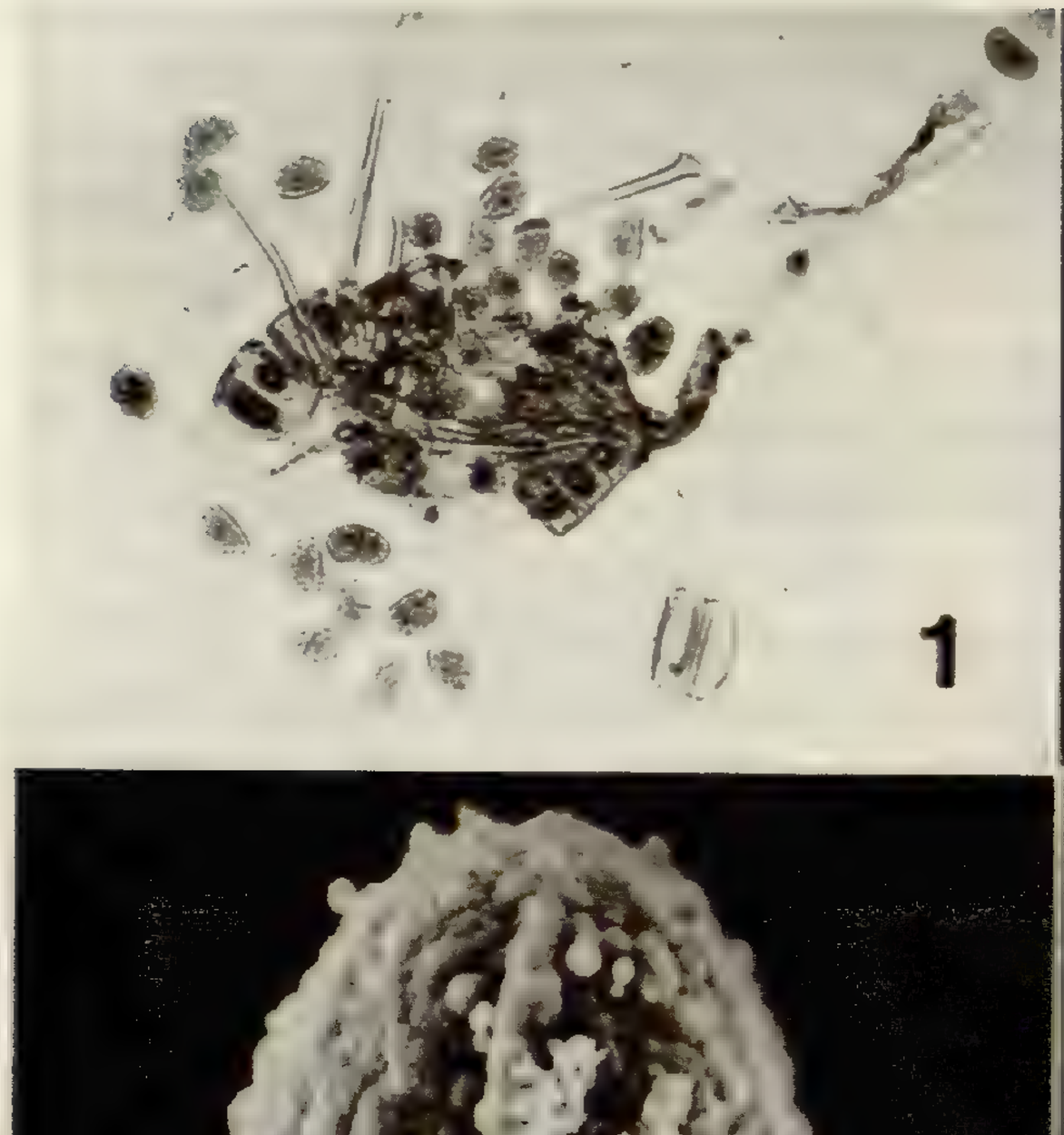
Most of the grammitid ferns, now placed by many authors in a separate family, Grammitidaceae, or a subfamily, Grammitidoideae, of the Polypodiaceae, were at one time classified as members of the genus *Polypodium*. In 1967 Morton wrote, "Evidence . . . has been accumulating in recent years that there are two major groups of species—those centering around the true *Polypodium*, as typified by *P. vulgare* L., and those belonging to *Grammitis* Swartz and some closely related small genera." Ching (1940) created a family, Grammitaceae, to accommodate these dwarf polypodies. In 1947 Holttum recognized the family, which he revised to Grammitidaceae, and later wrote (1955), "There are no intermediates between them and any true Polypodiaceae, and a close relationship seems very doubtful." After treating them, in 1947 and previously, as members of the Polypodiaceae, Copeland came to believe the group should have family status (1951). In her revision of *Grammitis* in New Guinea, Parris (1983) placed the genus in Grammitidaceae and gave a history of the family classification. Most authors have accorded the grammitid ferns family status, but W. Wagner (1973) retained them in a subfamily, the Grammitidoideae of the Polypodiaceae, and Tryon and Tryon (1982) placed them in a tribe, the Grammitideae of the Polypodiaceae.

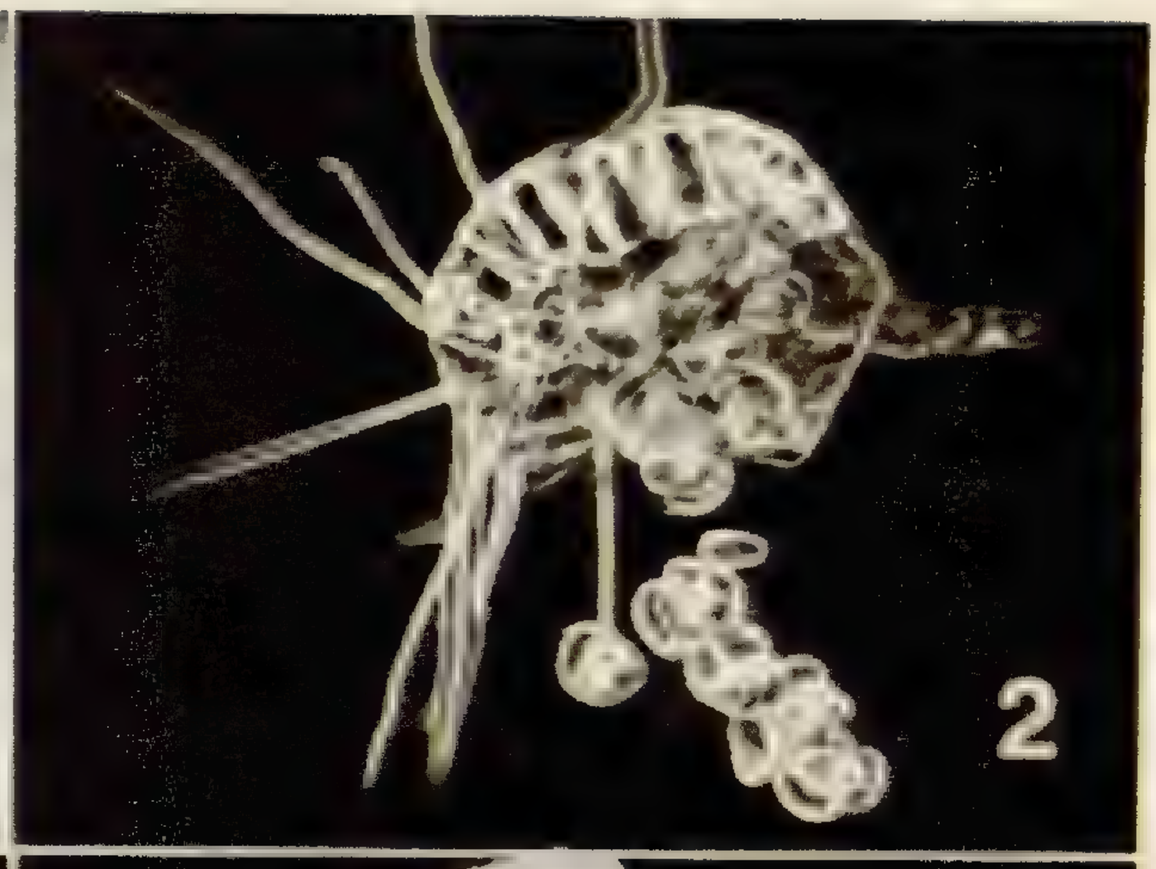
The degree of relationship between the grammitid and the polypodioid ferns still remains, however, a matter of argument. Several presumably clear-cut characters separate the two groups. These have been discussed by de la Sota (1960) and Morton (1967). Evans (1969) gave a comparison of the Grammitidaceae with the *Polypodium pectinatum-plumula* complex, and Price (1983) contrasted *Polypodium*, *Pecluma*, and *Ctenopteris*, the first two in the Polypodiaceae s.s., the last in the Grammitidaceae.

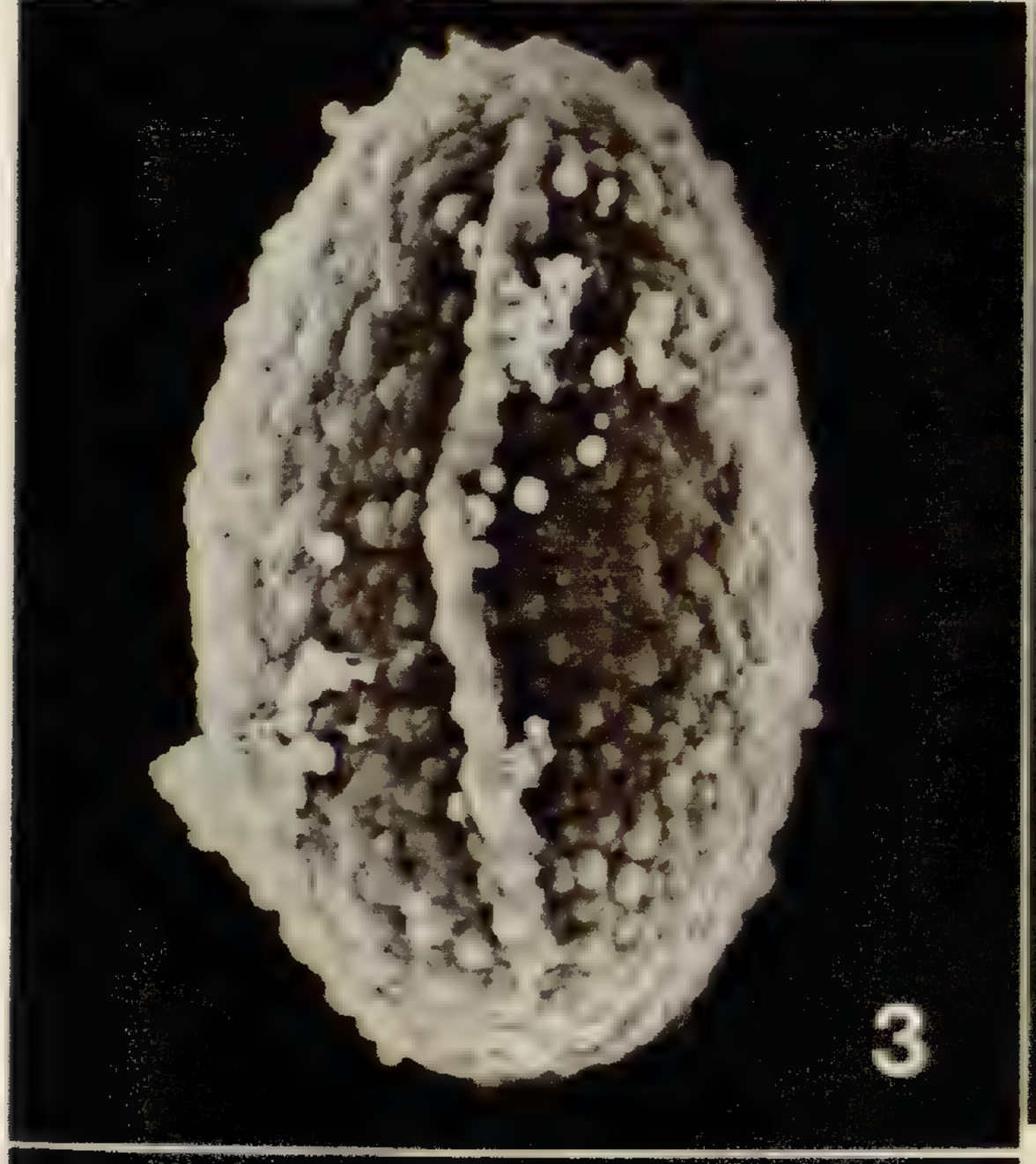
Briefly the distinctions between the polypodioid and the grammitid ferns are these: The Polypodiaceae s.s. have in general creeping rhizomes with scattered fronds, the grammitids have in general upright rhizomes and tufted fronds. Fronds of polypodies are often articulate, of grammitids, non-articulate. Scales of the former tend to be peltate and commonly borne on the leaf blades; those of the latter are basally attached and found only on the rhizome. The polypodioids bear scales and various types of hairs while the grammitid indument is typically composed of bristle-like setae often borne in pairs or multiples. Venation in the

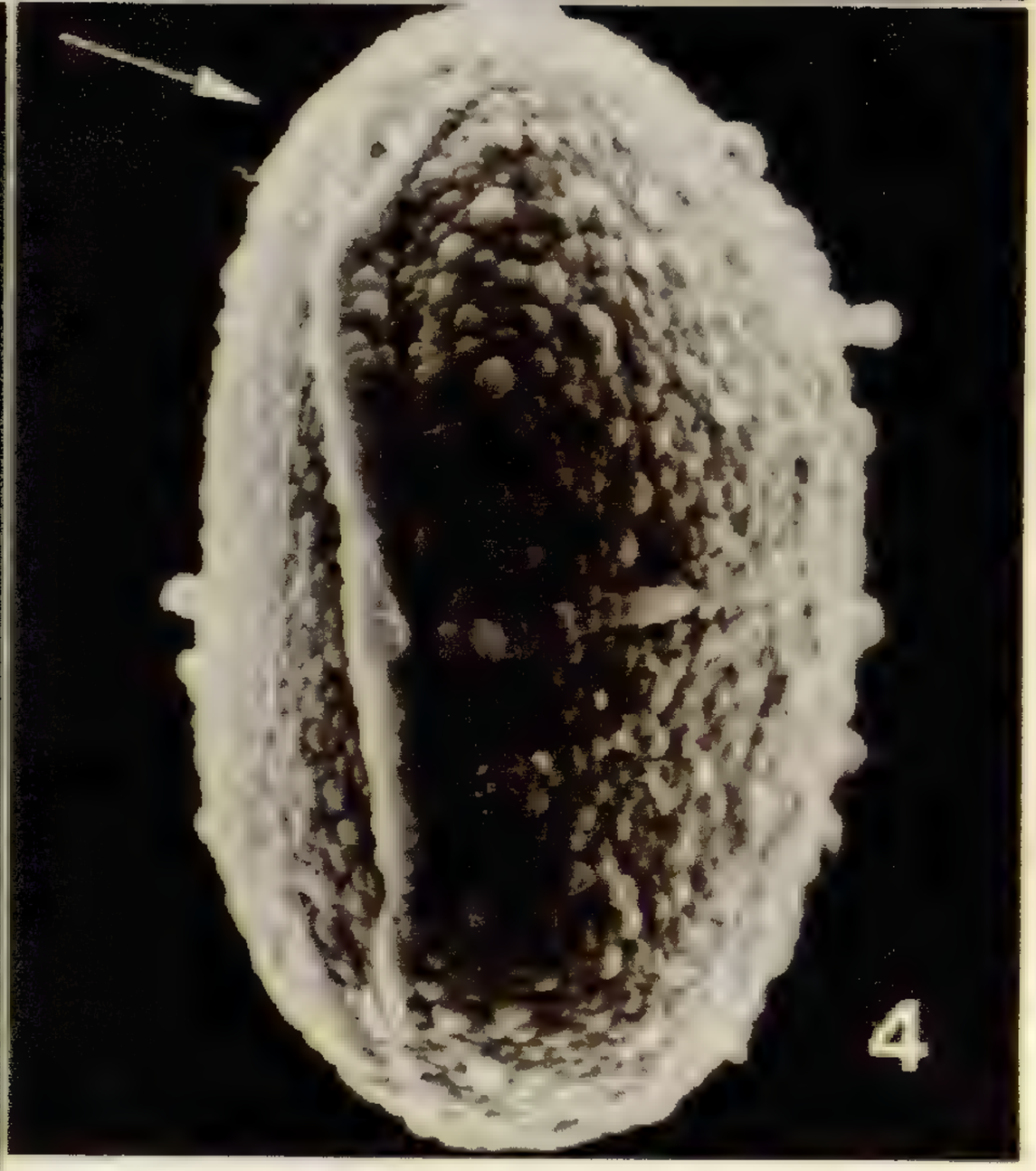
→

FIGS. 1–6. 1. Sporangium and spores of *Ctenopteris longa* (*Buchtien 5252*, MICH) × 80. 2. Sporangium and spores of *Grammitis lanigera* var. *lanigera* (*Wagner 78528*, MICH) × 80. 3–4. Scanning electron micrographs of spores of *G. lanigera* var. *lanigera* (*Wagner 77062A*, MICH) × 2500. Arrow in Figure 4 indicates fork in laesura. 5. Scanning electron micrograph of *G. lanigera* var. *stella* (*Little 9356*, US) × 2500. 6. *G. lanigera* var. *lanigera* (*Wagner 77512*, MICH) × ⅛.


1


2


3


4

5

6

polypodioids is characteristically reticulate with free included veinlets; in grammitids the veins are usually free, or if reticulate, without included veinlets. The sporangial stalk in the polypodioids is composed of two or three rows of cells, in the grammitids only one. Spores of polypodioids are bilateral, monolete, and without chlorophyll; spores of grammitids are tetrahedral to globose, trilete, and have chlorophyll. Although exceptions may be cited to many of the above characters, spores have traditionally provided a dependable difference: "Possibly the most uniform and easily observable difference is in the spores, those of *Polypodium* being monolete and those of *Grammitis* trilete . . ." (Morton, 1967). Exceptions to this rule are the subjects of this paper.

Between 1977 and 1983 collections made in Costa Rica in connection with a cytological survey of the ferns of that country, included several specimens of *Grammitis lanigera* (Desv.) Morton var. *lanigera* [*Ctenopteris lanigera* (Desv.) Copel.]. In all characters this fern appears to be an incontrovertible member of the Grammitidoideae. However the correlation of characters is not perfect. The exception is the spores. These are conspicuously bilateral (Figs. 2–4). They are green with tuberculate surface and a single long laesura, which commonly has a characteristic bend or slight angle near the center (Fig. 3). In some spores the laesura forms an abbreviated fork (Fig. 4, arrow).

The sporangium bears setae and has a stalk composed of a single row of cells for most of its length (Fig. 2). The plants are tufted epiphytes with a short rhizome (Fig. 6). Spreading, stiff, one-celled hairs are borne on the stipe and blade, and the rhizome scales are basally attached and setose. *Grammitis lanigera* var. *lanigera* has been recorded from Costa Rica to Bolivia and has also been found in Martinique and Hispaniola (Morton, 1967). Our collections are from the following localities in Costa Rica (all are W. Wagner numbers deposited in MICH): Alajuela, Road N of San Ramón, *77062A*; Cartago, Estrella, *79036*; Tapantí, *83012*; Heredia, Cerro Vueltas, *77008, 77013*; Slopes of Volcán Barba above Porrosatí, *77512*; Zurquí, *83019*; Puntarenas, Monteverde, *83004B*; San José, Cascajal, *78524, 78528*.

Other grammitid ferns suggesting relationship to *Grammitis lanigera* were subsequently examined to determine their spore shape. One of these is *Ctenopteris stella* Copel., which Morton (1967) considered to be a variety of *G. lanigera*. A specimen from Colombia (Huila, 15 km SE of Garzon, *Little 9356*, US) and one from Bolivia (Cochabamba, *King* and *Bishop 9677*, MO) were found to have bilateral green spores, but with a different laesural pattern. In these the majority have a modified trilete scar (Fig. 5). Furthermore, the sporangial stalk differs from that of the type variety, and from nearly all grammitid ferns, in having two rows of cells for most of its length. Thus, Morton has combined as varieties in one species, two ferns with very different sporangial stalks.

Similar confusion has been found in plants named *Grammitis cultrata* (Willd.) Proctor from Guatemala (Quezaltenango, Aguas Jorgines, *Skutch 904*, MICH) and Mexico (Chiapas, La Independencia, *Breedlove 33641*, MICH; Volcán de Tacana, *Matuda 2912*, MICH) all of which have a single-rowed sporangial stalk, while other specimens from the last locality (*Matuda S-225* and *S-229*, MICH) have two-rowed sporangial stalks. These differ also in the shape of the pinnae

which are shorter and more rounded in the last two collections. All of them have green bilateral spores and setose sporangia.

The same combination of characters, bilateral green spores and a two-rowed stalk together with a setose sporangium is found in two other grammitids, *Ctenopteris sericeolanata* (Hook.) Copel., which Morton thought to be synonymous with *Grammitis lanigera* var. *lanigera*, and *G. senilis* (Fée) Morton [*Ctenopteris senilis* (Fée) Copel.] from Central America, northern South America, and the Antilles, which Stolze (1981) placed in synonymy with *G. cultrata*.

Whether or not a single cell is to be found at the base of the two-rowed sporangial stalk in these ferns has not been determined. In *Prosaptia contigua* (Forster) Presl (Grammitidaceae) and in *Dictymia* J. Smith (Polypodiaceae) the sporangial stalk of two rows of cells is supported by a single cell. These are anomalies of uncertain significance in both families (Wilson, 1959). As a result of these observations, a broader consideration of polypodioid characters in grammitid ferns will be treated in a future paper.

Morton listed *Polypodium alternifolium* Hook. and *P. longum* C. Chr. [both are *Ctenopteris longa* (C. Chr.) Copel. according to Copeland, 1956] as two other species synonymous with *Grammitis lanigera* var. *lanigera*. A specimen of *P. alternifolium* (Ecuador, *Sodiro*, Aug. 1875, MICH), however was found to have tetrahedral spores. *Ctenopteris longa* (Bolivia, hacienda Simaco, camino a Tipuani, *Buchtien 5252*, MICH), on the other hand, has bilateral green spores like those found in our Costa Rican specimens (Fig. 1). Copeland (1956) in placing *P. longum* under *Ctenopteris* wrote: "Spores of South American specimens oblong; of Costa Rican, probably tetrahedral, but very few seen." He also described, as a new species, *Ctenopteris fabaespora* from Panama, basing the epithet on its bilateral spores. I have not seen a specimen, but according to David Lellinger (pers. comm.) Morton considered this fern to be the same as *Grammitis lanigera* var. *lanigera*. References, such as Copeland's, to bilateral spores in grammitid ferns are rare and have usually referred to the genus *Loxogramme*. Bilateral spores are known in *Loxogramme* in about half of the species, and although the genus has been thought by many to be a grammitid fern, evidence now seems to indicate that its relationships lie more closely with the polypodioid ferns (M. Price, pers. comm.).

Another suggestion that bilateral spores have been found in grammitids is that of Nayar and Devi (1965) who described the spores of *Ctenopteris brevivenosa* (v.A.v.R.) Holtt. as trilete-tetrahedral but added that many are monolete-bilateral. They also found *Prosaptia contigua* to have monolete-bilateral spores. Specimens of both these species (Singapore, *Holttum 23319*, MICH, and Sumatra, *H. H. Bartlett 7963*, MICH, respectively) were examined by me and neither was found to have bilateral spores. Thus there may be different spore types within the same species, or, perhaps, confusion about spore shape in certain Grammitidoideae may arise because of the widespread phenomenon of intrasporangial germination that occurs in this group of ferns (Stokey and Atkinson, 1958). At least three of the spores visible in my Figure 1 have divided. Sporangia are seen frequently with the entire contents at various stages of spore germination. Minute two-celled gametophytes, often with the spore wall still

TABLE 1. Selected Grammitid (G) and Polypodioid (P) Characters in Certain Grammitid Ferns.

	Spore			Sporangium	
	Color	Shape	Laesura	Capsule	Stalk
Grammitis lanigera var. *lanigera*	green[G]	bilateral[P]	monolete[P] with a bend	setose[G]	1 row[G] of cells
Grammitis lanigera var. *stella*	green[G]	bilateral[P]	modified[?] trilete	setose[G]	2 rows[P] of cells
Ctenopteris longa	green[G]	bilateral[P]	monolete[P] with a bend	setose[G]	1 row[G] of cells
Ctenopteris sericeolanata	green[G]	bilateral[P]	monolete[P]	setose[G]	2 rows[P] of cells
Grammitis cultrata	green[G]	bilateral[P]	monolete[P]	setose[G]	2 rows[P] of cells
Grammitis "cultrata"	green[G]	bilateral[P]	monolete[P] with a bend	setose[G]	1 row[G] of cells
Grammitis senilis	green[G]	bilateral[P]	monolete[P]	setose[G]	2 rows[P] of cells
Polypodium alternifolium	green[G]	tetrahedral[G] globose	trilete[G]	setose[G]	1 row[G] of cells

remaining, may appear oblong, when, in fact, they may have been formed from tetrahedral spores, and also swelling prior to division may make bilateral spores appear to be globose.

Variability in spore type and the occurrence of the two types of spores have been discussed by W. Wagner (1974). Within the same genus or very closely related genera, both bilateral and tetrahedral spores are known, for example, in *Gleichenia*, *Antrophyum*, *Vittaria*, and *Lindsaea*, as well as in *Loxogramme*. The situation in the grammitid ferns reported here is of special interest with regard to the relationships of the Grammitidaceae with the Polypodiaceae. All of the taxa discussed above have typical grammitid characters with important exceptions (Table 1). The occurrence of polypodioid characters suggests the possibility that the two groups of ferns may be more closely related than many authors have surmised. This study suggests, furthermore, that a re-evaluation of the taxonomy of this group of grammitid ferns is needed. Taxa that have been treated as synonymous or as varieties differ in characters that have usually been considered fundamental and conservative in the general taxonomy of ferns.

I am grateful to the following people for help in many ways: David Bay, Bruce Donahoe, Luis Diego Gómez, David Lellinger, and Warren H. Wagner. Material used in this study was made available through NSF (INT 7819909), the Organization for Tropical Studies, and three herbaria, MICH, MO, and US.

LITERATURE CITED

CHING, R. C. 1940. On natural classification of the family "Polypodiaceae." Sunyatsenia 5:201–268.
COPELAND, E. B. 1947. *Genera filicum*. Waltham, Mass.: Chronica Botanica.

———. 1951. *Grammitis*. Philipp. J. Sci. 80:93–271.
———. 1956. *Ctenopteris* in America. Philipp. J. Sci. 84:381–475.
EVANS, A. M. 1969. Interspecific relationships in the *Polypodium pectinatum-plumula* complex. Ann. Missouri Bot. Gard. 55:193–293.
HOLTTUM, R. E. 1947. A revised classification of leptosporangiate ferns. J. Linn. Soc., Bot. 53:123–158.
———. 1955. *A revised flora of Malaya*. Vol. II. *Ferns of Malaya*. Singapore: Government Office.
MORTON, C. V. 1967. The genus *Grammitis* in Ecuador. Contr. U.S. Natl. Herb. 38:85–123.
NAYAR, B. K. and S. DEVI. 1965. Spore morphology of Indian ferns. Grana Palynol. 6:121–127.
PARRIS, B. S. 1983. A taxonomic revision of the genus *Grammitis* Swartz (Grammitidaceae: Filicales) in New Guinea. Blumea 29:13–222.
PRICE, M. G. 1983. *Pecluma*, a new tropical American fern genus. Amer. Fern J. 73:109–116.
SOTA, E. R. DE LA. 1960. Polypodiaceae y Grammitidaceae Argentinas. Opera Lilloana 5:5–229.
STOKEY, A. G. and L. R. ATKINSON. 1958. The gametophyte of the Grammitidaceae. Phytomorphology 8:391–403.
STOLZE, R. G. 1981. Ferns and fern allies of Guatemala. Part II. Polypodiaceae. Fieldiana, Bot. n.s. 6:1–522.
TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.
WAGNER, W. H. 1973. Some future challenges of fern systematics and phylogeny. Bot. J. Linn. Soc. 67(Suppl. 1):245–256.
———. 1974. Structure of spores in relation to fern phylogeny. Ann. Missouri Bot. Gard. 61:332–353.
WILSON, K. A. 1959. Sporangia of the fern genera allied with *Polypodium* and *Vittaria*. Contr. Gray Herb. 185:97–127.

REVIEW

"Ferns to know and grow," by F. Gordon Foster. xiv + 228 pp. ISBN 0-917304-98-5. Portland, Oregon: Timber Press. 1984. $29.95 (hardbound).

Third revised and somewhat enlarged edition of *The gardener's fern book*, reviewed in Amer. Fern J. 56:81, 1966. This edition is in a larger page format and additional species are treated. Unfortunately, the quality of reproduction of the photographs has suffered. It may be ordered from Timber Press, P.O. Box 1631, Beaverton, OR 97075.—*A.R.S.*

American Fern Journal 75(1):12–18 (1985)

The Atomic Composition of *Onoclea sensibilis* Spores

RANDY WAYNE and PETER K. HEPLER
Department of Botany, University of Massachusetts, Amherst, MA 01003

It is generally accepted that fern spores contain all the elements that are needed for germination because spores germinate on distilled water in the absence of added nutrients (Raghavan, 1980). Here we present the first quantitative data on the atomic composition of *Onoclea* spores that permit one to know which elements are present in the spore. The presence of an element does not imply its essentiality as a nutrient; rather, it allows for the possibility that this element could be involved with development.

One element of particular interest to us is calcium, which is essential for spore germination (Wayne & Hepler, 1984a). Ninety percent of the calcium present in *Onoclea* spores resides in their walls, whereas 10% is intracellular (Wayne & Hepler, 1984b). By selectively removing calcium from the spores, we show that the wall-associated calcium is required for *Onoclea* germination in calcium-free media.

MATERIALS AND METHODS

Mature sporophylls of *Onoclea sensibilis* L. were collected in Amherst and Pelham, Massachusetts in January, 1981 and February, 1982 and stored in plastic bags in the freezer at −15°C. Prior to an experiment, sporangia were wetted with a 0.1% solution of Aerosol O. T. (Fisher Scientific Co., Pittsburgh, PA), sterilized for 1 min with 1 l of a 20% (v/v) solution of commercial bleach (5.25% NaOCl), and rinsed with 500 ml of sterile water in a manner modified from Stockwell and Miller (1974). For germination assays, approximately 2 mg of sterilized spores were sown on either 10 ml of the standard medium containing 1 mM $Ca(NO_3)_2$, 0.81 mM $MgSO_4$, and 3.45 mM KNO_3 (pH 5.2), or on 10 ml of 2.5 mM EGTA (ethylene glycol-bis(β-amino-ethyl ether)-N,N,N′,N′-tetraacetic acid, pH 6.8) for 24 h then transferred to 10 ml of the standard medium plus or minus $Ca(NO_3)_2$ one hour prior to irradiation. Spores were irradiated to induce germination for 5 minutes with broad band red light (energy fluence rate = 2.4 $J \cdot m^{-2} \cdot s^{-1}$). All manipulations were carried out in sterile plasticware in the dark or under a dim green safelight (Wayne & Hepler, 1984a).

Percent germination was determined 48 h after irradiation by the acetocarmine-chloral hydrate method of Edwards and Miller (1972). Data are expressed as the mean ± two standard errors of the mean.

In order to analyze the elements contained in the spores, sterilized spores were dried at 105°C for 2 h and weighed immediately. For the determination of calcium, magnesium, potassium, cobalt, nickel, iron, copper, manganese, sodium, zinc, mercury, cadmium, chromium, silver, molybdenum, vanadium, and lead,

spore samples were dissolved in hot concentrated sulfuric acid (1 ml) plus 10 drops of concentrated nitric acid, diluted with water to a 25–50 ml volume depending on weight (usually 10 mg), and then analyzed for metals using a Perkin-Elmer 403 Atomic Absorption Spectrophotometer following the Perkin-Elmer standard methods guide. Carbon, hydrogen, and nitrogen analyses were performed on a Perkin-Elmer 240 Elemental Analyser according to the modified Pregl-Dumas technique. Sulfur analysis was done by classical $BaSO_4$ titration using Thorin indicator after Schöniger oxygen flask combustion. Phosphorus was analyzed by forming phosphomolybdic acid from orthophosphate after conversion by hot H_2SO_4 and HNO_3 digestion. Phosphomolybdic acid was then measured spectrophotometrically at a wavelength of 882 nm. Oxygen analysis was done by a modified Unterzaucher procedure. Chlorine was analyzed as Cl^- after a Schöniger oxygen flask combustion followed by a coulometric titration with silver.

Culcita coniifolia, Cycas taiwaniana, Nothoscordum bivalve, Psilotum nudum, Selaginella kraussiana, and *Tradescantia virginiana* were grown in the greenhouse of the University of Massachusetts, Amherst. Voucher specimens are on deposit in the University of Massachusetts Herbarium, Amherst. *Funaria hygrometica* was grown in Laetsch's medium under continuous white light (Laetsch, 1967).

Results and Discussion

The atomic composition of spores of *Onoclea sensibilis* is shown in Table 1. The spores contain all the macro- and micronutrients needed for higher plant growth (Clarkson & Hanson, 1980). The relative atomic composition of *Onoclea* spores is similar to that reported for leaves of a variety of ferns (Höhne & Richter, 1981) and corn plants (Epstein, 1972). The high levels of minerals found in *Onoclea* spores contrast with the very low levels found in corn microspores (Pfahler & Linskins, 1973). The difference in the mineral content of corn microspores and *Onoclea* spores may reflect the functional differentiation of fern spores as propagules. *Onoclea* spores may carry sufficient nutrients to ensure the proper development of the gametophyte, whereas the style provides the nutrition for angiosperm microgametophytes. Consistent with their role as propagules, the spores can withstand considerable desiccation (cf. Barker & White, 1964) as evidenced by their low water content. In *Onoclea*, the dry weight accounts for 95.43 ± 1.02% of the fresh weight of the spore, whereas the dry weight of a plant cell may be only 7% of the fresh weight (Burling & Jackson, 1965; Ray, 1962).

Carbon, hydrogen, and oxygen are the most common elements in the spores. The carbon/hydrogen/oxygen molar ratio of 1/1.6/0.3 is a result of the abundance of storage lipids and proteins in the spores (DeMaggio & Stetler, 1980; Towill & Ikuma, 1975). This ratio remains nearly constant for 24 h following hydration. The carbon/hydrogen/oxygen ratio changes to 1/1.3/0.9 in week-old gametophytes indicating that in this later developmental stage carbon is mainly present as carbohydrate (Table 2).

Potassium, magnesium, sodium, and calcium are the major cations in the spores.

TABLE 1. The Atomic Composition of *Onoclea sensibilis* Spores.

Element	Percent dry weight	nmol/mg dry weight	Number of atoms relative to calcium
C	58.59 ± 2.00	48,784.35	977.64
O	21.25 ± 1.90	13,281.75	266.17
H	7.76 ± 0.55	76,942.16	1541.93
N	4.59 ± 0.28	3277.00	65.67
P	0.82 ± 0.18	254.74	5.31
K	0.70 ± 0.00	179.04	3.59
S	0.53 ± 0.07	163.76	3.28
Mg	0.34 ± 0.07	139.89	2.80
Na	0.23 ± 0.07	100.04	2.00
Ca	0.20 ± 0.07	49.90	1.00
Cl	0.110 ± 0.020	31.027	0.622
Co	0.040 ± 0.037	6.787	0.136
Fe	0.024 ± 0.006	4.297	0.086
Ni	0.011 ± 0.020	1.874	0.038
Pb	0.010 ± 0.018	0.483	0.010
Mn	0.008 ± 0.002	1.456	0.029
Zn	0.006 ± 0.003	0.918	0.018
Cu	0.005 ± 0.003	0.787	0.016
Cr	0.002 ± 0.000	0.385	0.008
Ag	0.002 ± 0.001	0.185	0.004
Cd	0.0004 ± 0.0006	0.035	0.0007
Hg	0.00001 ± 0.00001	0.0005	0.00001
V	<0.001	<0.196	<0.004
Mo	<0.0007	<0.073	<0.001
Unknown	4.8		
Total	100.0		

Calcium has been shown to be essential for the normal spore development. It is required for germination (Wayne & Hepler, 1984a), rhizoid growth (Miller et al., 1983), and normal protonema development (Cooke & Racusen, 1982). Calcium is also required for archegonium, antheridium, and sporophyte formation in *Woodsia obtusa* (Bryan & O'Kelley, 1967), and spermatogenesis in *Marsilea* (Wick, 1979). In *Onoclea*, the amount of calcium in the spores is similar to the levels of calcium found in the fiddlehead, expanded leaf, rachis, and empty sporangia; however, there is considerably more calcium found in gametophytes growing in a calcium-sufficient medium (Table 3). The average calcium content in the various tissues of *Onoclea* is 0.24% which is similar to that found in a variety of greenhouse-grown plants (Table 3).

Calcium, primarily localized in spore walls, is necessary for germination (Wayne & Hepler, 1984b). *Onoclea* spores, for example, germinate readily when placed in deionized water plus $MgSO_4$ and KNO_3, but not if they are prewashed with EGTA, a chelating agent that removes the wall bound calcium. The ability to germinate can be regained by adding exogenous calcium (Table 4), indicating the importance of external calcium. The potential of unwashed spores to germinate in calcium-free media depends on the spore density. We have found that

TABLE 2. The Carbon, Hydrogen, and Oxygen Ratios of Several Developmental Stages of *Onoclea*.

Developmental stage	Element	Atomic content (μmol/mg dry wt)	Ratio with respect to carbon
Dry spores	C	48.78 ± 1.66	1
	H	76.94 ± 5.46	1.6
	O	13.28 ± 1.19	0.3
Hydrated spores (dark)	C	48.40 ± 1.77	1
	H	81.56 ± 3.37	1.7
	O	15.00 ± 0.13	0.3
Hydrated spores (5 min red)	C	49.07 ± 1.03	1
	H	81.61 ± 1.49	1.7
	O	13.91 ± 1.44	0.3
Gametophytes (1 week old)	C	34.03 ± 3.36	1
	H	44.55 ± 2.56	1.3
	O	29.75 ± 9.75	0.9

germination is promoted by high spore density, probably as a result of pooling sufficient calcium from many cell walls (Wayne & Hepler, 1984a). Other studies also show that plants are capable of adding nutrients to "nutrient free," media thereby masking the requirement for a given nutrient (Miller et al., 1983; Pfahler & Linskins, 1973; Saunders & Hepler, 1983; Wayne & Hepler, 1984a).

The concentrations of the transition metals cobalt, nickel, lead, chromium, silver, and copper are relatively high (cf. Gauch, 1972). These trace elements may accumulate in the spores in order to provide a reservoir for the growing

TABLE 3. The Calcium Content of Plant Tissues.

Species	Tissue or organ	Calcium content (% dry weight)
Onoclea sensibilis	gametophyte	0.46
	empty sporangia	0.26
	rachis	0.22
	spore	0.20[a]
	expanded leaf	0.17
	fiddlehead	0.11
Onoclea sensibilis	average of all parts	0.24
Psilotum nudum	frond	0.06
Culcita coniifolia	frond	0.12
Selaginella kraussiana	shoot	0.42
Cycas taiwaniana	leaf	0.47
Nothoscordum bivalve	leaf	0.52
Funaria hygrometica	protonema	0.64
Tradescantia virginiana	leaf	1.10[b]

[a] The range is 0.1–0.4% dry weight.
[b] The high calcium content of *T. virginiana* is a consequence of the presence of calcium oxalate crystals.

TABLE 4. The Effect of Washing with EGTA on Germination of *Onoclea*.

Treatment	Percent germination
Control	89.00 ± 1.00
EGTA-washed	0.75 ± 1.50
EGTA-washed + 1 mM $Ca(NO_3)_2$	87.25 ± 4.50

gametophyte and young sporophyte before a sufficiently large root system can be produced. Alternatively, the accumulation of these heavy metals may be fortuitous but not essential (especially in the case of cobalt), indicating that *Onoclea* may be a bio-accumulator of heavy metals from the environment and thus may serve as a bio-indicator. Other ferns have been shown to accumulate heavy metals from the environment (see Puckett & Burton, 1981, for a review; Hunter, 1953). This would provide yet a new way in which a fern can be utilized as an environmental monitor (Klekowski & Poppel, 1976; Petersen et al., 1980; Petersen & Francis, 1980). Interestingly, many of these bivalent metals can act as calcium antagonists (Hagiwara, 1973) and thus as inhibitors of germination (Petersen et al., 1980; Petersen & Francis, 1980; Wayne & Hepler, 1984a).

The concentration of chloride is too low for it to serve as the only counter ion for potassium. The positive charges are probably neutralized by organic acids such as malic acid, which would account for the low intracellular pH we have observed in *Onoclea*.

The high level of phosphorus is probably due to the presence of phytic acid (inositol hexaphosphate) in the spores (DeMaggio et al., 1983). Inositol polyphosphate is usually considered to be a storage form of phosphate, although recently it has been shown to interact with calcium ions in triggering cell responses (Rasmussen, 1981).

Silicon is present in high concentrations in the spores of *Selaginella* (Tryon & Lugardon, 1978) and the leaves of many true ferns (Höhne & Richter, 1981). It may also be part of the spores of *Onoclea*. We, however, were unable to assay for it.

Here we have presented the natural abundance of elements in the spores of *Onoclea*. We have shown how the C/H/O ratio in particular reveals the developmental state of the cells and that there is sufficient calcium associated with the cells to facilitate germination. By removing the wall-bound calcium, we have also shown that the germination response requires external calcium. A bio-inorganic chemical analysis of spores complements other methods of analysis such as light (Vogelmann & Miller, 1980) and electron (Bassel et al., 1981) microscopy, biochemical studies (Huckaby & Miller, 1984; Towill & Ikuma, 1975; DeMaggio & Stetler, 1980) as well as physiological investigations (Vogelmann et al., 1981; Wayne & Hepler, 1984a), in understanding *Onoclea* spores and their development.

Elemental analyses were performed by Dr. G. Dabkowski, Director of the Microanalysis Laboratory, University of Massachusetts, Amherst. Support for this research was provided in part by NSF grant PCM 840 2414 to P.K.H. and a Sigma Xi grant in aid of research to R.W.

Literature Cited

Barker, W. G. and R. G. White. 1964. Retention of viability in lyophilized spores of the fiddlehead fern, *Matteuccia pensylvanica*. Amer. Fern J. 54:87–89.

Bassel, A. R., C. C. Kuehnert, and J. H. Miller. 1981. Nuclear migration and asymmetric cell division in *Onoclea sensibilis* spores: an ultrastructural and cytochemical study. Amer. J. Bot. 68:350–360.

Bryan, A. L. and J. C. O'Kelley. 1967. The influence of replacing calcium with strontium on the development of *Woodsia obtusa*. Amer. Fern J. 57:27–31.

Burling, E. and W. T. Jackson. 1965. Changes in calcium levels in cell walls during elongation of oat coleoptile cells. Pl. Physiol. 40:138–141.

Clarkson, D. T. and J. B. Hanson. 1980. The mineral nutrition of higher plants. Annual Rev. Pl. Physiol. 31:239–298.

Cooke, T. J. and R. H. Racusen. 1982. Cell expansion in the filamentous gametophyte of the fern *Onoclea sensibilis* L. Planta 155:449–458.

DeMaggio, A. E. and D. Stetler. 1980. Storage products in spores of *Onoclea sensibilis* L. Amer. J. Bot. 67:452–455.

———, T. Templeman, J. Soldatis, and D. A. Stetler. 1983. Ultrastructure and biochemistry of fern spore germination: protein and phytic acid. (Abstr.) Pl. Physiol. 72(Suppl.):99.

Edwards, M. F. and J. H. Miller. 1972. Growth regulation by ethylene in fern gametophytes. III. Inhibition of spore germination. Amer. J. Bot. 59:458–465.

Epstein, E. 1972. *Mineral nutrition of plants: principles and perspectives*. New York: John Wiley & Sons.

Gauch, H. G. 1972. *Inorganic plant nutrition*. Stroudsburg, Pa.: Dowden, Hutchinson, & Ross.

Hagiwara, S. 1973. Ca spike. Adv. Biophys. 4:71–102.

Höhne, H. and B. Richter. 1981. Untersuchungen über den Mineralstoff- und Stickstoffgehalt von Farnkrautern. Flora 171:1–10.

Huckaby, C. S. and J. H. Miller. 1984. Spore germination and rhizoid differentiation in *Onoclea sensibilis*. Pl. Physiol. 74:656–662.

Hunter, J. G. 1953. The composition of bracken: some major- and trace-element constituents. J. Sci. Food Agric. 4:10–20.

Klekowski, E. J., Jr. and D. M. Poppel. 1976. Ferns: potential in situ bioassay system for aquatic borne mutagens. Amer. Fern J. 66:75–79.

Laetsch, W. M. 1967. Ferns. In *Methods in developmental biology*, eds. F. H. Wilt and N. K. Wessells. New York: Thomas Y. Crowell.

Miller, J. H., T. C. Vogelmann, and A. R. Bassel. 1983. Promotion of fern rhizoid elongation by metal ions and the function of the spore coat as an ion reservoir. Pl. Physiol. 71:828–834.

Petersen, R. L., D. Arnold, D. G. Lynch, and S. A. Price. 1980. A heavy metal bioassay based on percent germination of the sensitive fern, *Onoclea sensibilis*. Bull. Environm. Contam. Toxicol. 24:489–495.

——— and P. C. Francis. 1980. Differential germination of fern and moss spores in response to mercuric chloride. Amer. Fern J. 66:75–79.

Pfahler, P. L. and H. F. Linskins. 1973. Ash percentage and mineral content of maize (*Zea mays* L.) pollen and style. Theor. Appl. Genet. 45:32–36.

Puckett, K. J. and M. A. S. Burton. 1981. The effect of trace elements on lower plants. Pp. 213–238 in *Effect of heavy metal pollution on plants*, vol. 2, ed. N. W. Lepp. London: Applied Science Publishers.

Raghavan, V. 1980. Cytology, physiology and biochemistry of germination of fern spores. Int. Rev. Cytol. 62:69–118.

Rasmussen, H. 1981. *Calcium and cAMP as synarchic messengers*. New York: Wiley.

Ray, P. M. 1962. Cell wall synthesis and cell elongation in oat coleoptile tissue. Amer. J. Bot. 49: 928–939.

Saunders, M. J. and P. K. Hepler. 1983. Calcium antagonists and calmodulin inhibitors block cytokinin-induced bud formation in *Funaria*. Developm. Biol. 99:41–49.

Stockwell, C. R. and J. H. Miller. 1974. Regions of cell wall expansion in the protonema of a fern. Amer. J. Bot. 61:375–378.

TOWILL, L. R. and H. IKUMA. 1975. Photocontrol of the germination of *Onoclea* spores. IV. Metabolic changes during germination. Pl. Physiol. 56:468–473.

TRYON, A. F. and B. LUGARDON. 1978. Wall structure and mineral content in *Selaginella* spores. Pollen & Spores 20:315–340.

VOGELMANN, T. C., A. R. BASSEL, and J. H. MILLER. 1981. Effects of microtubule-inhibitors on nuclear migration and rhizoid differentiation in germinating fern spores (*Onoclea sensibilis*). Protoplasma 109:295–316.

——— and J. H. MILLER. 1980. Nuclear migration in germinating spores of *Onoclea sensibilis*: the path and kinetics of movement. Amer. J. Bot. 67:648–652.

WAYNE, R. and P. K. HEPLER. 1984a. The role of calcium ions in phytochrome-mediated germination of spores of *Onoclea sensibilis* L. Planta 160:12–20.

——— and P. K. HEPLER. 1984b. Red light stimulates an increase in intracellular calcium in the spores of *Onoclea sensibilis*. Pl. Physiol. 77:8–11.

WICK, S. M. 1979. The localization and possible role of calcium ions during mitosis. Dissertation, Stanford University.

REVIEW

"*Gametophytes of Ophioglossaceae*," by D. D. Pant, D. D. Nautiyal, and D. R. Misra. Phyta Monograph 1:1–111, 1984.

This is a report on the gametophytes and young embryos of the Ophioglossaceae in India. Six species of *Ophioglossum*, 4 species of *Botrychium*, and *Helminthostachys* were studied from extensive collections of gametophytes. A helpful introduction to the literature initiates this conventional morphological study. Observations on the structure and development of these gametophytes and embryos expand our knowledge about the gametophytes from India. New and interesting information presented includes: meristems of the gametophyte lobes of *Helminthostachys* that suggest that the lobes are short lateral branches; slight lignification of the central strand of elongated parenchyma cells in the cylindrical gametophytes of *Ophioglossum* and *Helminthostachys*; absence of a ventral canal cell in *Ophioglossum* archegonia; and endoscopic embryo development in *O. nudicaule*. The concluding section of the monograph summarizes the new observations and incorporates them into a useful review of the literature. Pertinent information from the literature is also tabulated in a 12 page table at the end of the text. There is an abundance of illustrations (over half of the monograph). The line drawings are excellent but the halftones are in need of improvement. The quality of the halftones is not a serious detraction from the paper because much of the illustrated material is presented as both line drawings and halftones. "*Gametophytes of Ophioglossaceae*" is an informative paper that will be of interest to pteridologists, especially those interested in the morphology and anatomy of gametophytes. This monograph may be purchased from the Society of Indian Plant Taxonomists, Department of Botany, University of Allahabad, Allahabad 211002, India.—DEAN P. WHITTIER, Department of Biology, Vanderbilt University, Nashville, TN 37235.

American Fern Journal 75(1):19–21 (1985)

Ethnopteridology of the Chácobo Indians in Amazonian Bolivia

BRIAN M. BOOM
New York Botanical Garden, Bronx, NY 10458

The Chácobo are a small tribe of some 400 Indians belonging to the Panoan language family who live in an isolated rainforest region in northern Bolivia. During a six-month ethnobotanical study of the Chácobo living in the vicinity of the village Alto Ivon, a special effort was made to collect all the pteridophytes and to obtain indigenous names and information on use.

The village of Alto Ivon (11°45′S, 66°02′W) is located some 60 air miles south of Riberalta in the Bolivian department of Beni. The forest is Tropical Moist with a distinct dry season, generally from about June to October. The elevation is about 200 m and the terrain is level. The forest canopy is at about 25 m, and the species composition appears to be typical of southwestern Amazonia. The five ecologically most important tree families, as revealed by a 1 ha. inventory, are Moraceae, Myristicaceae, Palmae, Leguminosae sensu lato, and Vochysiaceae. This tree flora is not especially species-rich; only 94 species with a DBH greater than 10 cm were encountered in the hectare.

As with the trees, the pteridophyte flora is not particularly rich. Between Nov. 1983 and Apr. 1984, 13 genera and 25 species of pteridophytes were collected. If the species diversity was not impressive, what was quite interesting was the high degree of fern utilization. The Chácobo have uses for 16 species, or 65 percent of the total encountered in their territory. The uses are all medicinal in nature and generally involve the making of a decoction of fronds or rhizomes. No instances of ferns used as food were encountered.

In Table 1 an accounting is given of the ferns collected, the indigenous names, and the manner in which they are employed. Numbers listed are in the author's series, and vouchers are deposited at NY. Specimens were collected in the forest surrounding Alto Ivon, brought back to the village, and presented to Chácobo informants to elicit names and uses. Ten principal informants were used: Pae Chávez, Caco Soría, Yari Vargas, Tani Chávez, Cana Antelo, Pae Ortíz, Pae Davalos, María Soría, Caco Ortíz, and Rabi Ortíz. The last individual was the tribal chief at the time of the study and was responsible for transcribing my tape recorded interviews. When more than one name is given for a species, this represents the name elicited from a second informant and not an alternative name provided by the same informant. A guide to the pronunciation of names is given in Appendix 1.

I could not detect any general term comparable to "fern" in English. Chácobo fern names often encompass several botanical species (Table 2). Most of the names appear to be complex terms made up of common terms having other applications in the Chácobo language. Thus, *cashimëtsisi* can be broken down into *cashi* (= bat, the mammal) and *mëtsisi* (= claws); perhaps this alludes to the

TABLE 1. Ferns of the Chácobo Indians.

Adiantopsis radiata (L.) Fée: *mitaisa* (*5048*), not used.

Adiantum glaucescens Kl.: *mitaisi* (*4813*), frond decoction used to bathe children who have high fever; *xëqui nihijahëhua* (*4997*), not used.

Adiantum lucidum (Cav.) Sw.: *mitaisi* (*4704*), frond decoction used to bathe children who have high fever; *mitahitsa* (*5010*), not used; *xëquitaxo* (*4090*), frond decoction drunk to cure rheumatism.

Adiantum obliquum Willd.: *xëquihuitaxo* (*4076*), frond decoction drunk to cure diarrhea; *xëquitaxo* (*4085*), frond decoction drunk to cure rheumatism.

Adiantum petiolatum Desv.: *xëquihuitaxo* (*4077*), frond decoction drunk to cure diarrhea.

Adiantum tomentosum Kl.: *mitaisa* (*5061*), not used.

Asplenium pearcei Baker: *mitaisa jahëhua* (*5051*), not used; *jonotasi* (*4127*), frond decoction drunk to cure stomachache.

Ctenitis protensa (Afz.) Ching: *toriahuitaxo* (*4812*), rhizome shavings boiled and cooled decoction drunk to cure appendicitis.

Ctenitis submarginalis (Langsd. & Fisch.) C. Chr.: *toriahuitaxo* (*4998*), rhizome shavings placed directly on skin over appendix to relieve pain of appendicitis; *jinaristi* (*4124*), frond decoction drunk to alleviate headache.

Cyathea sp.: *capëtëjiquërëxë* (*4088*), frond decoction drunk to relieve pain of appendicitis.

Hecistopteris pumila (Spreng.) J. Smith: no Chácobo name (*4403*), not used.

Lindsaea divaricata Kl.: *toriahuitaxo* (*4730*), rhizome shavings boiled and cooled decoction drunk to cure appendicitis.

Lindsaea lancea (L.) Brade var. *lancea*: *xëqui jahuëhua* (*4911*), frond decoction used to bathe hyperactive children.

Lindsaea portoricensis Desv.: *mitaisa* (*5049*), not used.

Lomagramma guianensis (Aubl.) Ching: no Chácobo name (*4743, 4799*), not used.

Lomariopsis japurensis (Mart.) J. Smith: *toriahuitaxo* (*5020*), not used; *cashimëtsisi* (*4089*) two drops of stipe exudate mixed with water and this solution drunk to alleviate stomachache.

Metaxya rostrata (Willd.) Presl: *xëqui jahuë* (*5047*), not used.

Polypodium bombycinum Maxon: *jihui ratsamica nishi* (*5036*), rhizome scrapings macerated in cold water and resulting paste is put directly on skin over appendix to cure appendicitis.

Polypodium (*Campyloneurum*) aff. *brevifolium* Link: *jonotasi* (*4128*), frond decoction drunk to cure stomachache.

Polypodium (*Phlebodium*) *decumanum* Willd.: *jonotasi* (*4889*), not used.

Polypodium (*Microgramma*) *megalophyllum* Desv.: *cashimëtsisi* (*4019*), frond decoction drunk to alleviate stomachache.

Polypodium sp.: *mitaisa* (*5050*), *xëqui jahëhua* (*4380*), not used.

Thelypteris (*Goniopteris*) *abrupta* (Desv.) Proctor, vel aff.: *xëqui jahuëhua* (*5014*), not used.

Trichomanes pinnatum Hedw.: *jorojina* (*4640*), not used; *mitahuisma* (*4130*), frond decoction drunk to cure diarrhea.

Trichomanes vittaria Poir.: *jorojina* (*4023*), reportedly used as a medicinal, but informant could not be more specific.

small curved scales on the rhizome. Other names, like *mitaisi*, appear to be simple, proper nouns applying only to those particular species. Of course, it is possible that *mitaisi* has a non-fern meaning unknown to me or to the linguists of the Summer Institute of Linguistics who have studied the language and have prepared a dictionary (unpublished). A translation of name components is given in Table 2.

Appreciation is extended to Drs. John T. Mickel and Alan R. Smith for identifying the specimens, to Dr. Brent Berlin for reviewing the manuscript, and

TABLE 2. Chácobo Fern Names.

capëtëjiquërëxë [*capëtë* (cayman); *ji* (line, tail); *quërëxë* (arrow head with several barbs)]—*Cyathea* sp.
cashimëtsisi [*cashi* (bat, the mammal); *mëtsisi* (claws, fingernails)]—*Lomariopsis japurensis*; *Polypodium* (*Microgramma*) *megalophyllum*
jihui ratsamica nishi [*jihui* (tree); *ratsamica* (to climb, grasping tightly); *nishi* (liana)]—*Polypodium bombycinum*
jinaristi [*jina* (line, tail)]—*Ctenitis submarginalis*
jonotasi—*Asplenium pearcei*; *Polypodium* (*Campyloneurum*) aff. *brevifolium*; *Polypodium* (*Phlebodium*) *decumanum*
jorojina [*jina* (line, tail)]—*Trichomanes pinnatum*; *Trichomanes vittaria*
mitahitsa—*Adiantum lucidum*
mitahuisma—*Trichomanes pinnatum*
mitaisa—*Adiantopsis radiata*; *Adiantum tomentosum*; *Lindsaea portoricensis*; *Polypodium* sp.
mitaisa jahëhua [*jahëhua* (mother)]—*Asplenium pearcei*
mitaisi—*Adiantum glaucescens*; *Adiantum lucidum*
toriahuitaxo—*Ctenitis protensa*; *Ctenitis submarginalis*; *Lindsaea divaricata*; *Lomariopsis japurensis*
xëqui huitaxo [*xëqui* (maize)]—*Adiantum obliquum*; *Adiantum petiolatum*
xëqui jahuë [*xëqui* (maize); *jahuë* (thing, something)]—*Metaxya rostrata*
xëqui jahuëhua [*xëqui* (maize); *jahuëhua* (that which is larger than . . .)]—*Lindsaea lancea* var. *lancea*; *Polypodium* sp.; *Thelypteris* (*Goniopteris*) *abrupta*
xëqui nihi jahëhua [*xëqui* (maize); *nihi* (forest); *jahëhua* (mother)]—*Adiantum glaucescens*
xëqui taxo [*xëqui* (maize)]—*Adiantum lucidum*; *Adiantum obliquum*

especially to the Edward John Noble Foundation for a generous grant that made fieldwork amongst the Chácobo possible.

APPENDIX 1. Pronunciation of Chácobo names.

After studying the Chácobo language, the Summer Institute of Linguistics devised a written form employing symbols that were as similar as possible to Spanish. Under their system there are 21 symbols in the Chácobo alphabet. These are listed below with my interpretation of their Spanish or English equivalent sound.

a = Spanish *a*. **b** = Spanish *b*. **c** = Spanish *c*. **ch** = Spanish *ch*. **ë** = no exact equivalent; intermediate in sound to the Spanish *u* and *e*; unrounded midcentral vowel as in the English word *hurt*. **h** = no exact equivalent; similar in sound to a "hard" English *h*, executed with a glottal stop. **hu** = no exact equivalent; similar to the English sound *wh*. **i** = Spanish *i*. **j** = Spanish *j*. **m** = Spanish *m*. **n** = Spanish *n*. **o** = Spanish *o*. **p** = Spanish *p*. **qu** = no exact equivalent; similar in sound to the English *kw*. **r** = Spanish single-trill *r*. **s** = Spanish *s*. **sh** = no exact equivalent; similar in sound to the English *sh*. **t** = Spanish *t*. **ts** = no exact equivalent; similar in sound to the English *ts*, executed with a strong oral explosive. **x** = no exact equivalent; similar in sound to the English *sh*. **y** = Spanish *y*.

American Fern Journal 75(1):22–28 (1985)

Special Report

The Present Evolutionary and Taxonomic Status of the Fern Genus *Polystichum*: The 1984 Botanical Society of America Pteridophyte Section Symposium

DAVID S. BARRINGTON
Department of Botany, University of Vermont, Burlington, VT 05405

The fern genus *Polystichum* has presented major problems in definition and circumscription of species since its description by Roth in 1799. Part of the problem is the vast diversity within the genus: the number of species is reported as more than 175 by Copeland (1947). In addition, hybridization is extremely common (Knobloch, 1976), and agamospory has been reported among experimental plants from Europe (Vida & Reichstein, 1975). There is also substantial evidence of phenotypic and ontogenetic variability within species of the genus. Good progress has been made in solving evolutionary and taxonomic problems in the north-temperate and boreal regions (Manton, 1950; Manton & Reichstein, 1961; Kurata, 1964; Sleep & Reichstein, 1967; Daigobo, 1972; W. Wagner, 1973; D. Wagner, 1979); however, little taxonomic and virtually no evolutionary work has been done on the genus in tropical or austral regions. Hence this large genus of dryopteroid ferns is in need of substantial attention from systematic and evolutionary biologists, especially in tropical regions.

Christopher Haufler at the University of Kansas organized a symposium on *Polystichum* for the American Institute of Biological Sciences meetings in Fort Collins, Colorado, during August of 1984. As Haufler noted in his opening remarks, the purpose of the symposium was to draw attention to a complex and poorly understood genus of ferns, rather than to solve problems by consensus of the speakers. Participants in the symposium focused on the evolutionary problems encountered in New World *Polystichum*, primarily from a taxonomic and floristic viewpoint. This paper summarizes the Fort Collins symposium.

MORPHOLOGICAL VARIATION AND EVOLUTION IN *POLYSTICHUM*

Warren H. Wagner, Jr.—University of Michigan

The systematic problems in the genus *Polystichum* are best placed in the context of the structural variation evident at different taxonomic levels. The genus as a whole is typical of the Polypodiales in having a suite of diagnostic features none of which is universal: terrestrial habit; medium stature; short-creeping and scaly rhizome; lustrous, coriaceous, once- to twice-pinnate, free-veined, anadromous blades; pinna (pinnule) margins more or less spinulose;

indusium abaxial and peltate; spores bilateral and monolete; perispore well-developed; haploid chromosome number of 41 or a multiple thereof. Transformation of diagnostic features (such as loss of the peltate indusium) has led to problems in definition of *Polystichum* as well as the description of various segregate genera, which may or may not represent monophyletic groups. In *Polystichum*, as in many fern genera, definition of a group and its component species has been based on subjective arguments such as those from tradition, authority, and consistency—rather than from arguments based on objective documentation and analysis of the variation in structure throughout the group.

Several of the genera segregated from *Polystichum* are in need of critical re-evaluation. *Plecosorus*, known from Mexico to Panama, differs only in lacking a true indusium (common in *Polystichum*) and having a modified, recurved margin protecting the sporangia (false indusium). *Papuapteris* of New Guinea shares both of the critical features of *Plecosorus*, presumably as homoplasies. New World taxa placed in *Phanerophlebia* or *Cyrtomium* have broad lamina segments provided with irregularly anastomosing veins (not with regularly anastomosing veins with included veinlets as in true Old World *Cyrtomium*) but are otherwise typical members of *Polystichum*. Hybrids of other genera and *Polystichum* are now known. Most dramatic is the hybrid between *Dryopteris goldiana* and *Polystichum lonchitis* from Ontario, on which the University of Michigan pteridologists are currently working. *Polystichum* and its allied genera constitute an array of evolutionary groups that have maintained at least partial genetic homology in spite of isolation and independent evolution.

Hybridization and polyploidization commonly generate reticulate complexes in *Polystichum*. However, a series of plants that appear to be hybrids may actually be the products of divergence. One newly documented complex from Hawaii comprises a group of three bipinnate species that are similar in leaf dissection and general aspect, but differ in chromosome number ($n = 41$, 82, and 164), leaf and segment shape, habitat preference, and scale structure.

Within species, structural features can be highly variable. Numerous trivial forms of the well-known eastern North American *Polystichum acrostichoides* have been reported and described. A peculiar environmental factor has been pinpointed as responsible for the derivation of forma *incisum* (Gray) Gilbert, which has variously pinnatifid pinnae instead of typical serrulate pinnae on leaves produced late in a season on damaged plants bearing otherwise typical foliage (W. Wagner et al., 1970). Such striking short-term alterations demonstrate that, in *Polystichum*, variations may be superficial and should be tested.

Polystichum in Temperate and Boreal North America

David H. Wagner—University of Oregon

Polystichum is depauperate in eastern North America (four species, one of which is endemic), but more diverse in the West (13 species, nine of which are endemic). A hypothesis for a reticulate complex involving 11 of the species is based on an analysis of the sexual species and seven known sterile hybrids (W. Wagner, 1973; D. Wagner, 1979). From the seven basic diploid species, five tet-

raploids and one hexaploid have been generated in nature. All but one of the six needed diploid (or tetraploid) progenitors are known. Though fragmentary specimens presumed to represent the sixth, a second progenitor for *P. andersonii*, are on hand, better documentation (complete plants, cytological analysis, or hybrid synthesis) is needed.

Despite the current good resolution of the complex, individual specimens are frequently difficult to identify from diagnostic keys. The difficulty may be better understood with reference to Vavilov's "Law of Homologous Series in Variation" (Vavilov, 1922). Based on exhaustive studies of crop plants, Vavilov concluded that closely allied species are characterized by similar and parallel series of hereditary variations. In *Polystichum*, parallel series of hereditary variations are evident in ontogenetic series among the species with divided fronds. For example, a juvenile frond of *P. braunii* (fully bipinnate at maturity) is less divided than a mature frond of *P. setigerum*, which is merely bipinnatifid at maturity. When plants are under environmental stress, leaf development is arrested in a juvenile, stunted condition, although sori may still be produced. For example, the tetraploid *Polystichum scopulinum* growing in exposed sites at high elevations closely mimics the tetraploid *P. kruckebergii*, with which it shares a set of chromosomes contributed by the diploid *P. lemmonii*. Hybrid taxa, whether fertile species or sterile hybrids, approach parental extremes in variation when growing in a habitat typical of one parent.

The most nearly parallel series of variations is found in the two sibling species, *P. munitum* and *P. imbricans*. Although consistently ecologically distinct, the two are often found in contiguous habitats and hybridize frequently. The hybrids are sterile, as shown by cytological analysis. Continuing studies of these two species should be of great interest, since our understanding of divergent speciation in the ferns lags behind our understanding of polyploidization as a route to new species.

Polystichum in the West Indies

John T. Mickel—New York Botanical Garden

West Indian *Polystichum* is the most distinctive element in the New-World *Polystichum* flora. Nevertheless, it has received attention only twice: Maxon's (1909, 1912) classic revisionary work and Morton's (1967) study of the Cuban species. Seventeen of the 30 known species are once pinnate, and 14 are proliferous; both of these traits are rare on the mainland (though common in Japan and the Himalayas). Cuba, Jamaica, and Hispaniola constitute the center for the West Indian group: 25 of the 30 species are endemic there, whereas only three of the species reach Puerto Rico and two the Lesser Antilles. Only two species, *P. muricatum* and *P. platyphyllum*, are found both in the West Indies and in continental Latin America. Both are widespread species of low and middle elevations. Many of the West Indian species are limited to calcareous rock and soils, which is doubtless a critical determinant in their geographic distribution.

Wherever *Polystichum* has been studied in detail, hybridization has been shown to play an important role in speciation. Preliminary study suggests that

the West Indian taxa are no exception. Chromosome counts have been made for only six species (Walker, 1966). However, analysis of gross structural features of the sporophytes, as well as of spore-surface structure and spore-shape irregularities, when taken together with the few cytological records, demonstrates that there are at least six sterile hybrids and four tetraploid nothospecies among West Indian *Polystichum*.

Polystichum in Mexico

Alan R. Smith—University of California, Berkeley

Recent floristic work has provided the first modern treatments of *Polystichum* for the southern limits of the North American continent (Smith, 1981; Stolze, 1981). The genus in northern Latin America comprises about sixteen species, mostly confined to southern Mexico. Four species groups can be discerned. The largest group, comprising nine species (all of which are bipinnate, indusiate, and grow at middle to high elevations) has its affinities with the *P. setiferum* group (section *Metapolystichum* of Daigobo, 1972). The second group is of two north-temperate species at the southern limits of their ranges: *P. munitum* from Isla Guadelupe and *P. acrostichoides* from Nuevo Leon. The third group has a single representative, the rare *P. munchii*, closely allied to the West Indian *P. echinatum*. Like many of its West Indian allies, *P. munchii* is limited to limy substrates. A fourth group of four species has affinities farther south in continental Latin America. Two, *P. polyphyllum* and *P. speciosissimum*, are exindusiate species from high elevations; the other two, *P. platyphyllum* and *P. mickelii*, occur in low to mid-elevation rain forests. Seven of the Mexican species have been examined cytologically. Four counts are diploid ($n = 41$); *P. fournieri* and *P. polyphyllum* are tetraploid ($n = 82$); and *P. platyphyllum*, probably a species complex, has both cytotypes. In northern Latin America, it appears that species infrequently grow together, and interspecific hybridization has not been documented.

Polystichum in Central and South America

David S. Barrington—University of Vermont

Polystichum in Central and South America can be divided into two geographical and evolutionary groups, one tropical and the other austral. The austral group has received some attention (Christ, 1893, 1905; Looser, 1968), but the tropical one has not until quite recently (Barrington, 1985).

The situation in Costa Rica is best known, where the genus is represented by six species above 2800 m. Two are at the southern limit of their range (diploid *P. speciosissimum* and tetraploid *P. fournieri*) and two others are common throughout the mountains of the New World tropics (diploid *P. lehmannii* and tetraploid *P. polyphyllum*). In addition, two species are apparently endemic, an undescribed diploid and an undescribed tetraploid. Three hybrids have been

found, including a sterile triploid (with 41 pairs and univalents) between the two endemic species and a sterile triploid between the endemic tetraploid and some unknown diploid progenitor allied to *P. polyphyllum*. The two endemic species appear to be an allotetraploid and one of its diploid progenitors based on chromosomal evidence, morphology, and preliminary electrophoretic analysis (C. H. Haufler, pers. comm.). Secondary interaction in the Sierra Talamanca of Costa Rica appears to be promoted by human disturbance, because the hybrids all occur in disturbed sites, and the progenitor species are found in proximity most often along roadcuts and in pastures and disturbed cloud forest and alpine areas.

Preliminary work in northern South America suggests that there are few species and that hybrids are not common. At high elevations, two broad-ranging species are common: *P. lehmannii* (along open streambanks) and *P. polyphyllum* (throughout the páramo). In upper-elevation cloud forests, there is a suite of locally endemic and mostly undescribed species (mostly from the moist margins of small streams). At middle elevations, the broad-ranging *P. platyphyllum* grows with the locally common *P.* (or *Cyrtomium*) *dubium*. In one mixed population of the two in Ecuador, there are plants with an array of features intermediate between *P. dubium* and *P. platyphyllum* that resemble the type of *P. dictyophyllum*. Spores of these intermediates are irregular, allowing the suggestion that at least some plants assignable to *P. dictyophyllum* are hybrids between *P. dubium* and *P. platyphyllum*. Further study of this trio should provide better understanding of the relationship of the anomalous *P. dubium* to the rest of *Polystichum*. At low elevations, the broad-ranging *P. muricatum* is found as far south as Ecuador. This diploid species is peculiar in that sori often abort before maturity, though regular spores are produced during meiosis. Reniform indusia at some sori are a second unusual feature of *P. muricatum*.

Summary

In the New World, there are five recognizable elements in *Polystichum*: the highly reticulated boreal–north temperate element (affinities circumboreal); the Mexican element (affinities warm–temperate Asian); the Antillean element (affinities in Southeast Asia and the western Pacific); the Andean element (affinities unclear); and the Austral or South Andean element (affinities austral, including New Zealand and Australia). All of these but the Austral element received some attention in this symposium; all need further scholarly attention. The symposium speakers noted in the discussion that followed the presentations that multiple origin of analogous character states is particularly common in *Polystichum*: for example, the true indusium has been lost in three of the five elements of the flora, the Antillean, the Andean, and the Austral.

Polystichum species, defined on both morphological and cytological grounds, have characteristic habitats and elevational preferences. Hybridization typically yields sterile hybrids and new species via polyploidization, both in north-temperate areas and in montane tropics. However, in the tropics, hybridization appears to be localized: the Greater Antilles and Costa Rica are rich in hybrids, whereas other parts of the Neotropics have not yet yielded many hybrids. Either

hybrids remain to be detected in these other regions, or further inquiry may allow a new understanding of what promotes hybridization in the genus.

All of the contributors to the symposium agreed that variability, especially ontogenetic and phenotypic but also in response to perturbation of individual plants, has played an important role in confusing specialists in the group. The usual duplication of names provided by specialists working in isolation on conspecific plants has been complicated by the peculiar reduction of the vast majority of bipinnate *Polystichum* species to a few taxa, initiated by Hooker (1862) and maintained by Christensen (1905–6).

Students of *Polystichum* have before them the substantial task of providing monographic treatments for tropical American groups. In addition, there remains the task of identifying phylogenetic groups above the species level among New World *Polystichum*.

Literature Cited

Barrington, D. S. 1985. The morphology and origin of a new *Polystichum* hybrid from Costa Rica. Syst. Bot. 10:199–204.

Christ, H. 1893. Les différentes formes de *Polystichum aculeatum* (L. sub *Polypodio*), leur groupement et leur dispersion, y compris les variétés exotiques. Ber. Schweiz. Bot. Ges. 3:26–48.

———. 1905. Uber die australen *Polystichum*-Arten. Ark. Bot. 4(12):1–5.

Christensen, C. 1905–6. *Index filicum*. Copenhagen: Hagerup.

Copeland, E. B. 1947. *Genera filicum*. Waltham, Mass.: Chronica Botanica.

Daigobo, S. 1972. Taxonomical studies on the fern genus *Polystichum* in Japan, Ryukyu, and Taiwan. Sci. Rep. Tokyo Bunrika Daigaku, Sect. B. 15(224):57–80.

Hooker, W. J. 1862. *Species filicum*. Vol. 4. London: W. Pamplin.

Knobloch, I. W. 1976. Pteridophyte hybrids. Publ. Mus. Michigan State Univ. Biol. Ser. 5:277–352.

Kurata, S. 1964. On the Japanese ferns belonging to the *Polystichum polyblepharum* group. Sci. Rep. Yokosuka City Mus. 10:17–41.

Looser, G. 1968. Los helechos del genero *Polystichum* Roth en Chile: notas preliminares. Anales del Museo de Historia Natural 1:49–58.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge Univ. Press.

——— and T. Reichstein. 1961. Zur Cytologie von *Polystichum braunii* (Spenner) Fée und seiner Hybriden. Ber. Schweiz. Bot. Ges. 71:370–383.

Maxon, W. R. 1909. A revision of the West Indian species of *Polystichum*. Studies in Tropical American Ferns 2. Contr. U.S. Natl. Herb. 13:25–38.

———. 1912. Further notes on the West Indian species of *Polystichum*. Contr. U.S. Natl. Herb. 16:49–51.

Morton, C. V. 1967. Studies of fern types. I. Contr. U.S. Natl. Herb. 38:29–83.

Roth, A. W. 1799. Auszug eines Briefes von Hrn. Prof. Mertens in Bremen an den Herausgeber. Arch. Bot. (Leipzig) 2(1):103.

Sleep, A. and T. Reichstein. 1967. Der Farnbastard *Polystichum meyeri* hybr. nov. = *Polystichum braunii* (Spenner) Fée × *P. lonchitis* (L.) Roth und seine Cytologie. Bauhinia 3:299–309, 363–374.

Smith, A. R. 1981. Part II. Pteridophytes. In *Flora of Chiapas*, ed. D. E. Breedlove. San Francisco: California Acad. of Sciences.

Stolze, R. G. 1981. Ferns and fern allies of Guatemala. Part 2. Polypodiaceae. Fieldiana, Bot. n.s. 6:1–522.

Vavilov, N. I. 1922. The law of homologous series in variation. J. Genet. 12:48–89.

Vida, G. and T. Reichstein. 1975. Taxonomic problems in the fern genus *Polystichum* caused by

hybridization. Pp. 126–135 in *European floristic and taxonomic studies*, ed. S. M. Walters. Faringdon: E. W. Classey.

WAGNER, D. H. 1979. Systematics of *Polystichum* in western North America and north of Mexico. Pteridologia 1:1–64.

WAGNER, W. H., JR. 1973. Reticulation in Holly Ferns (*Polystichum*) in the western United States and adjacent Canada. Amer. Fern J. 63:99–115.

————, D. R. FARRAR, and B. W. MCALPIN. 1970. Pteridology of the Highlands Biological Station area, southern Appalachians. J. Elisha Mitchell Sci. Soc. 86:1–27.

WALKER, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Trans. Royal Soc. Edinburgh 66:169–237.

SHORTER NOTES

***Marsilea quadrifolia* and *M. vestita* in the Floras of Kansas and Missouri.**— In the fern floras of both Kansas and Missouri two species of water-clover ferns, *Marsilea*, have been recognized: a native species, *Marsilea vestita* Hook. & Grev., and the introduced Eurasian *M. quadrifolia* L. *M. vestita* is common in central and western Kansas, while *M. quadrifolia* has been reported from only two counties in the southeastern part of the state (Petrik-Ott, Nova Hedwigia Beih. 61:1–332, 1979). In Missouri, both species have been reported from a single county each: *M. vestita* (as the synonym *M. mucronata* A. Braun) from Barton County in southwestern Missouri and *M. quadrifolia* from Platte County in northwestern Missouri (Steyermark, Flora of Missouri, Iowa State University Press, Ames, 1963). On the basis of specimens known at present, however, *Marsilea quadrifolia* should be deleted from the flora of Kansas and *M. vestita* should be deleted from the flora of Missouri. The latter is particularly of note as this species has been listed as endangered in Missouri.

Petrik-Ott (op. cit.) reported *M. quadrifolia* from Kansas on the basis of two specimens, both from the herbarium of the University of Kansas (KANU): *Kolstad & Harms 1581* from Cherokee Co., and *Holland 1993* from Neosho Co. The reasons for this are unclear as both sheets are annotated as *M. vestita* by Petrik-Ott, and the only mention of *M. quadrifolia* on either sheet is on the original label of *Holland 1993*. An additional specimen (*Harms 1137*, KANU) from Cherokee Co. was not cited by Petrik-Ott but was annotated by her as *M. quadrifolia*; this specimen has characters of sporocarp pedicel length (7 mm), position of attachment of this pedicel (4 mm above the petiole base), and glabrate sporocarps that are characteristic of *M. quadrifolia*. The specimen also has, however, asymmetrical abaxially hairy leaflets that are longer than wide and have concave inner margins and slightly crenulate terminal margins. In addition, the rhizomes are 1.0 mm or less thick and lack roots in the internodes. These are all characters of *M. vestita*, which normally has short-pedicelled (2–3 mm), basally attached hairy sporocarps.

The long sporocarp pedicel attached above the petiole base and the glabrous sporocarps in this plant are perhaps due to the development of the sporocarp under water, which has been shown to modify pedicel length and sporocarp indument (Bhardwaja, Trop. Ecol. 8:17–21, 1967). Sporocarps are normally produced only when these amphibious plants are emersed and dry, but occasionally the sporocarps develop under shallow water. In such instances the pedicel is attenuated and both the pedicel and sporocarp are almost glabrous. If the petiole is also attenuated, the pedicel, which is in fact a portion of that same leaf rather than the petiole of a separate leaf, may appear above the base of the petiole rather than at the base.

In Missouri, *Marsilea quadrifolia* was reported from Platte Co. by Gier (Amer. Fern J. 45:64–65, 1955) and was recollected there by Dunlap in 1962 (specimen in SMS). Steyermark (op. cit.) reported *M. vestita* from 1.5 miles north of Milford, Barton Co., on the basis of *Palmer 53101*. While I have not seen this particular specimen, I have seen others from the same locality: *Palmer 54495* (MICH), *Palmer 53949* (GH, KANU), *Dunlap* s.n. in 1961 (NLU, SMS), and *Dunlap* s.n. in 1963 (SMS). Of these, *Palmer 54495* and the Dunlap specimen from 1961 at NLU are fertile. Both bear the paired or triple (occasionally single) long-stalked sporocarps attached above the petiole base that characterize *M. quadrifolia*; in addition both these specimens and the sterile ones have symmetrical glabrous leaflets as wide as long or wider, with convex or straight inner margins and entire terminal margins, and rhizomes 1.0–1.3 mm thick bearing internodal roots, further confirming this identification.

A specimen of *Marsilea* from yet a third locality in Missouri was included among specimens sent from SMS: Boone Co., Watkins pond southwest of Midway, 12 June 1963, *Dunlap* s.n. Although this specimen is of a sterile, floating-leaved form, in which both species would have glabrous symmetrical entire-margined leaflets on long flexuous petioles, its stouter rhizomes and internodal roots indicate that it is also *M. quadrifolia*.

The criteria presented by Petrik-Ott (op. cit.) for distinguishing the two species are fairly reliable as long as adequate fertile material and leaves formed on land are present in a collection; the additional characters mentioned here are supplementary ones that can be used with scanty fertile or even sterile material, regardless of whether it grew on land or in water. Caution should be exercised in applying these characters outside the Great Plains region, however, as some of them, e.g., internodal roots, are found in extralimital species as well.

Marsilea quadrifolia appears to be spreading westward from northeastern North America, and collectors should continue to seek it both from additional localities in Missouri and from Kansas. *M. vestita*, although it is not expanding its range, is probably locally dispersed by migrating waterbirds and may eventually thus be brought into Missouri from nearby stations in eastern Kansas and Nebraska.

I thank the curators of GH, KANU, MICH, NLU, and SMS for making specimens available for this study, and R. Brooks, W. H. Wagner, Jr., and an anonymous reviewer for comments on the manuscript.—DAVID M. JOHNSON, Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

New Records for Longevity of *Marsilea* Sporocarps.—In 1952, Allsopp (Nature 169:79–80) reported the viability of *Marsilea* sporocarps 61.5 and 68 years old. These sporocarps were obtained from collections made by C. G. Pringle in Mexico in 1890 and by W. N. Suksdorf in Washington state in 1883. Recently, I successfully cultivated sporophytes from herbarium material 99–100 years old.

In fall of 1982, sporocarps of *Marsilea crenata* Presl collected by D. H. Campbell in the Philippines in 1911 (from the herbarium of the University of Michigan–MICH) were scarified and placed in water for a class demonstration. Young sporophytes from these 71 year-old sporocarps were apparent a few days later. Success with these sporocarps prompted me to try to obtain plants from sporocarps that were still older. In December 1983 I was successful in producing sporophytes of *M. vestita* Hook. & Grev. from a specimen on loan from Field Museum (F) collected by K. Brandegee in California in 1906 (77 years old) and of *M. mollis* Robinson & Fernald from a specimen (also on loan from F) collected by C. G. Pringle in Oaxaca, Mexico, in 1894 (89 years old). Finally, in March 1984 I obtained young plants from a collection on loan from Gray Herbarium (GH) of *M. oligospora* Goodding made by W. N. Suksdorf in 1883/1884. At 99 to 100 years of age these are the oldest sporocarps yielding sporophytes yet recorded in *Marsilea*. Voucher specimens of plants grown from these collections will be deposited in MICH and at the institutions that provided the viable spore material.

In view of reports of apomictic production of sporophytes in *Marsilea* (Bhardwaja & Abdullah, Nova Hedwigia 21:521–528, 1972), I thought it possible that these sporophytes were being produced directly from megagametophytes without fertilization, but isolated megaspores from the Brandegee, Pringle, and Suksdorf collections yielded no sporophytes while the controls did, and micro- and megagametophyte development appeared normal.

The maximum ages that viable *Marsilea* sporocarps are capable of reaching are yet to be determined, and it is thus valuable to have the abundant and widely distributed collections of such collectors as Pringle and Suksdorf for periodic sampling. Already, however, there is differential behavior among specimens. Only sporocarps from the Suksdorf collection at GH produced sporophytes; sporocarps from other herbaria, while they opened normally after scarification, showed neither gametophyte nor sporophyte development. This may represent natural loss of viability but may also be viability loss due to treatment with microwaves for fumigation (Hill, Taxon 32:614–615, 1983). As Hill (op. cit.) pointed out, having viable propagules present on herbarium sheets often makes it possible to obtain live plants of species otherwise difficult or expensive to collect. For *Marsilea* there is the added advantage that plants grown from specimens with mature sporocarps yield improved material for description and study; specimens with mature sporocarps usually have leaves in poor condition.

Recently, Bhardwaja (Aspects of Plant Sciences 3:39–62, 1980) reported the production of gametophytes (but not sporophytes) from *M. burchellii* A. Braun sporocarps 130 years old. In time perhaps this finding can be matched by the production of sporophytes from specimens equally old.

I thank the curators of F, GH, and MICH for making these specimens available

on loan, and W. H. Wagner, Jr., for comments on the manuscript.—DAVID M. JOHNSON, Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Nomenclatural Notes on some Ferns of Costa Rica, Panama, and Colombia.–II.—This is a continuation of the series begun a few years ago (Amer. Fern J. 67: 58–60. 1977) to record changes of names pertinent to ongoing floristic projects.

Alsophila imrayana var. **basilaris** (Christ) Lellinger, comb. nov.—*Cyathea basilaris* Christ, Bull. Herb. Boissier II, 4:949. 1904.—LECTOTYPE (chosen by Gastony, Contr. Gray Herb. 203:127, 1973): Costa Rica, *Wercklé* (P not seen; authentic material NY, US).

Glyphotaenium trifurcatum (L.) Lellinger, comb. nov.—*Polypodium trifurcatum* L., Sp. Pl. 2:1084. 1753.—TYPE: Based on t. 138 in Plumier's "Tractatus," which illustrates a specimen collected by Plumier on Martinique.

Thelypteris frigida (Christ) Smith & Lellinger, comb. nov.—*Aspidium frigidum* Christ, Bull. Herb. Boissier II, 6:160. 1906.—TYPE: Costa Rica, Pcia. Cartago, Volcán Turrialba, *Wercklé* (P not seen).

DAVID B. LELLINGER, Dept. of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560.

REVIEW

"*The ferns and fern allies of Southern Africa*," by W. B. G. Jacobsen. 542 pp. ISBN 0-409-09836-1. Durban, South Africa: Butterworth Publishers Ltd. 1983. R70.40 (ca $60.00).

The plan of this book is much like that of a college textbook, with parts, numbered sections, and shaded tables. There are two parts: Part I deals with ecology, environment, and distribution; Part II covers the pteridophytes in systematic order. The area covered includes not only South Africa, but all of southern Africa south of the 17th parallel.

The first part of the book is excellent—I have seen ecology textbooks with less detailed discussion of factors affecting distribution. Pteridophyte examples are used throughout this discussion of factors such as topographic (elevation), edaphic (geomorphology and soils), climatic (rainfall, temperature, slopes, light, wind), and life forms. For those of us in the northern hemisphere it helps to remember that southern Africa is sub-tropical since the tip is at 35° S (about the same as Los Angeles), and that north-facing slopes are warmer and drier.

The plant communities are described in a fairly long chapter. There is a list

of localities, dominant trees, undershrubs, vines, and in tabular form the rare, occasional, and frequent pteridophytes to be found in each. The photographs vary in quality, some giving a good representation of the forest type, fern, etc.; others are not as good.

The second part, dealing with the southern Africa Pteridophyta, begins with some general descriptive material on collecting and preserving specimens, and a glossary (an annoying place for this, in the middle of the book). Since I am not familiar with the fern flora, this part of the review concentrates on the more mechanical aspects of the book. There is both a technical key to the families, and a more descriptive key for families or genera. The keys throughout (including the keys to species of each genus) are a nuisance to use, and this is the only really objectionable part of the book. They are of the indented type, but not indented. So, for the first key, to make comparison "1," one reads through a vertical column of numbers: 1, 2, 3, 3′, 2′, 4, 4′, 2″ (help!), 1′, 5, 6, ... It is difficult enough to find and read the paired comparisons without having to worry about whether there is a third statement embedded in the column someplace. Fortunately there are not third choices very often as far as I could tell, but it takes a lot of unnecessary work to find each pair of statements. The characteristics used are clear, and the statements are generally well contrasted. There are keys to the genera within families and to species within genera. I tried a few where I had material available, and found no problems, but my material was from cultivated plants and not extensive enough to really evaluate the keys.

For each species there is a description, discussion of distribution and ecology, a photograph of an herbarium specimen, and a nice distribution map. There are a few line drawings, which are more helpful than the photographs. I found a few errors, but none of any real consequence: map 81 should be map 8 (p. 138), Mickel is several times spelled Mickels, Torres Street should be Torres Strait (p. 382), and a few others of even lesser consequence.

With the single exception of the keys, I liked the book. It would certainly be helpful if one were planning a trip to this fascinating part of the world, and I enjoyed reading the ecological section and thinking about going. The parts on ecology of pteridophytes, although referenced to southern Africa, have wide general application and make a nice introduction to this neglected area of pteridology.—JAMES D. MONTGOMERY, Ichthyological Assoc., Inc., Berwick, PA 18603.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (see cover 2).

A splendid four-color volume...

THE
FERN HERBAL

Including the Ferns, the Horsetails, and the Club Mosses

Written and illustrated by ELFRIEDE ABBE

This handsome volume is a reproduction of a now-rare limited edition of 150 copies which first appeared in 1981—the magnificent accomplishment of one gifted person, Elfriede Abbe, who was the author, illustrator, designer, typesetter, printer, and binder.

The text describes 27 members of the Filicales, covering their habitat and distribution, culture, history, and use.

Collectors of rare books, herbalists, fern enthusiasts, and other horticulturalists will want to own this fine volume. About the author: Elfriede Abbe was artist-in-residence in the Botany Department at Cornell before she retired. She has continued her career as an artist in Vermont where she has produced her own handprinted, handbound books on many subjects. Her artwork has been exhibited internationally. 70 wood engravings and drawings, including 20 full-color, full-page plates. $35.00

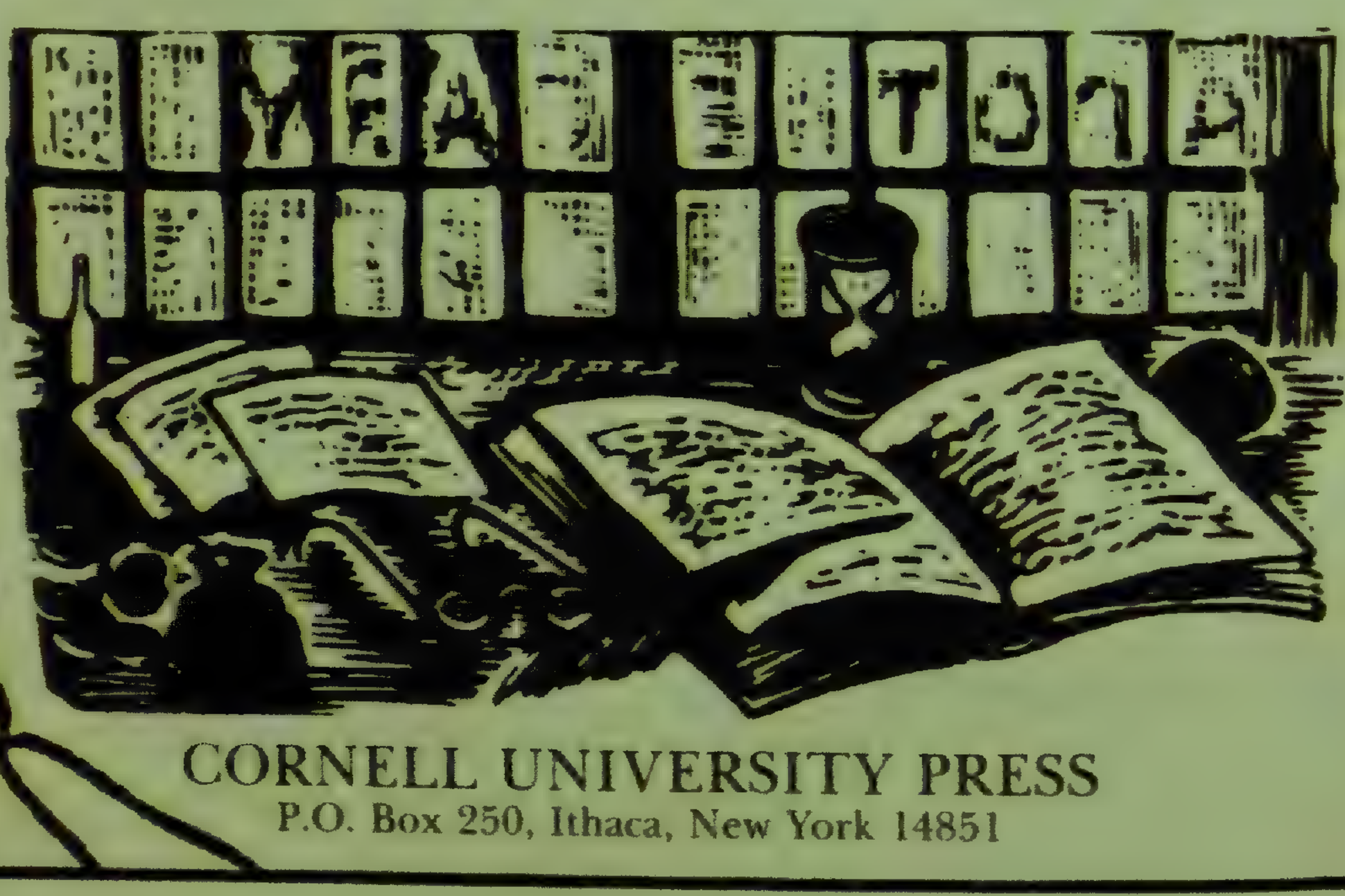
Also of interest—

GARDEN SPICE AND WILD POT-HERBS

An American Herbal

By W.C. MUENSCHER *and* MYRON A. RICE

With illustrations by ELFRIEDE ABBE $12.95 paper



CORNELL UNIVERSITY PRESS

P.O. Box 250, Ithaca, New York 14851

AMERICAN FERN JOURNAL

Volume 75

Number 2

April–June 1985

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Three New Anemias from Northern South America *John T. Mickel* 33

Sporopollenin Content of the Spore Apparatus of *Azolla*
R. E. Toia, Jr., B. H. Marsh, S. K. Perkins, J. W. McDonald, and *G. A. Peters* 38

Role of Morphological Characteristics of Leaves and the Sporangial Region in the Taxonomy of *Isoetes* in Northeastern North America *L. S. Kott* and *D. M. Britton* 44

New Species of *Thelypteris* from Puerto Rico *George R. Proctor* 56

Shorter Notes

***Dryopteris ludoviciana* and *D.* ×*australis* New to Arkansas**
James H. Peck, Steve L. Orzell, Eric Sundell, and *Carol J. Peck* 71

Polystichum *aleuticum* from Adak Island, Alaska, a Second Locality for the Species
David K. Smith 72

Review 37

The American Fern Society

Council for 1985

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268.
President

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109.
Vice-President

MICHAEL I. COUSENS, Faculty of Biology, University of West Florida, Pensacola, FL 32504.
Secretary

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Department of Botany, University of Vermont, Burlington, VT 05401.
Records Treasurer

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Barnard College, Columbia University, New York, NY 10027.
Fiddlehead Forum Editor

American Fern Journal

EDITOR

ALAN R. SMITH ..... Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER ..... Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER ..... U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER ... Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Jounal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VA 05401. Second-class postage paid at Burlington and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ichthyological Associates, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to Dr. David S. Barrington, Department of Botany, University of Vermont, Burlington, VT 05401.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $9.00 gross, $8.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $8.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $160.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

Fiddlehead Forum

Prof. Dennis Wm. Stevenson, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027, is editor of the newsletter "Fiddlehead Forum." The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcombed, and are tax-deductible. Inquiries should be address to the Secretary.

American Fern Journal 75(2):33–37 (1985)

Three New Anemias from Northern South America

JOHN T. MICKEL
New York Botanical Garden, Bronx, NY 10458

Of the over 100 species of *Anemia*, about 65 are found in southern and eastern Brazil with a secondary center of speciation in Mexico (20 species). Central America and the western and northern portions of South America have provided relatively few species, those being mostly very widespread taxa. Apparently this is a misleading picture since we here report three new species from northern South America—one from northern Brazil and two from essentially a single locality near Puerto Ayacucho in southern Venezuela. All three species are known from single collections and were found among granite rocks. It would seem that these areas have not been well collected, and since *Anemia* generally favors grassy and/or rocky habitats, there may be additional taxa yet to be discovered in southern Venezuela and Colombia and northern Brazil.

Anemia antrorsa Mickel, sp. nov. (Figs. 1G–I).—TYPE: Brazil, Amazonas, lower slopes of Pico Rondon, Perimetral Norte Highway Km 211, 3 km from Km 211, granite rock outcrop, 2 Feb 1984, *Prance et al. 28739* (holotype NY; isotype UC).

Anemiam tomentosam var. *australem* Mickel forma laminae simulans sed pinnis fertilibus brevibus antrorsis falcatis et laminae pilis contortis discreta. (L., *antrorsus*, directed upward, referring to the habit of the fertile pinnae.)

Rhizome horizontal, compact, ca. 6 mm diam., with brownish-yellow hairs, 5–8 mm long; fronds erect, sterile and fertile fronds alike, 28–34 cm long; stipe slightly less than half the sterile frond length and slightly more than half the fertile frond length, densely hirsute with orange hairs 3–5 mm long; blade deltate-ovate, 15–17 cm long, 8–10 cm broad, bipinnate-pinnatifid, lamina papyraceous, dull, pilose on both surfaces with multicellular hairs 0.5–1.0 mm long (twisted on abaxial surface), densely tomentose on rachillae, lower surfaces and rachis; sterile pinnae 9–12 pairs, subsessile (petiolules 1–2 mm), upper pinnae perpendicular to the rachis, lower pinnae slightly ascending, 5–7 pinnules per pinna, the lower pinnae slightly more exaggerated basiscopically, lobes acute to obtuse, margin entire to crenulate; veins free, evident; fertile pinnae remote from the sterile pinnae, ca. half as long as the adjacent sterile pinnae, ascending at 55° angle to strongly upwardly falcate, short-petiolulate (3–4 mm), the ultimate divisions with narrow laminar tissue; spores tetrahedral, vertically compressed, striate, the ridges broad with narrow grooves between, 69–79 (av. 76.1) μm diam. (Figs. 2A, B).

This species bears a strong resemblance to *A. tomentosa* var. *australis* Mickel, but *A. antrorsa* is quite distinct with its short, suberect pinnae, strongly hairy stipe and rachis, and twisted abaxial laminar hairs. Such habit for fertile pinnae is otherwise well known in the genus, occurring in all three subgenera: subg.

FIG. 1. Habit and details of three *Anemia* species. A–C. *Anemia ayacuchensis*. D–F. *Anemia porrecta*. G–I. *Anemia antrorsa*.

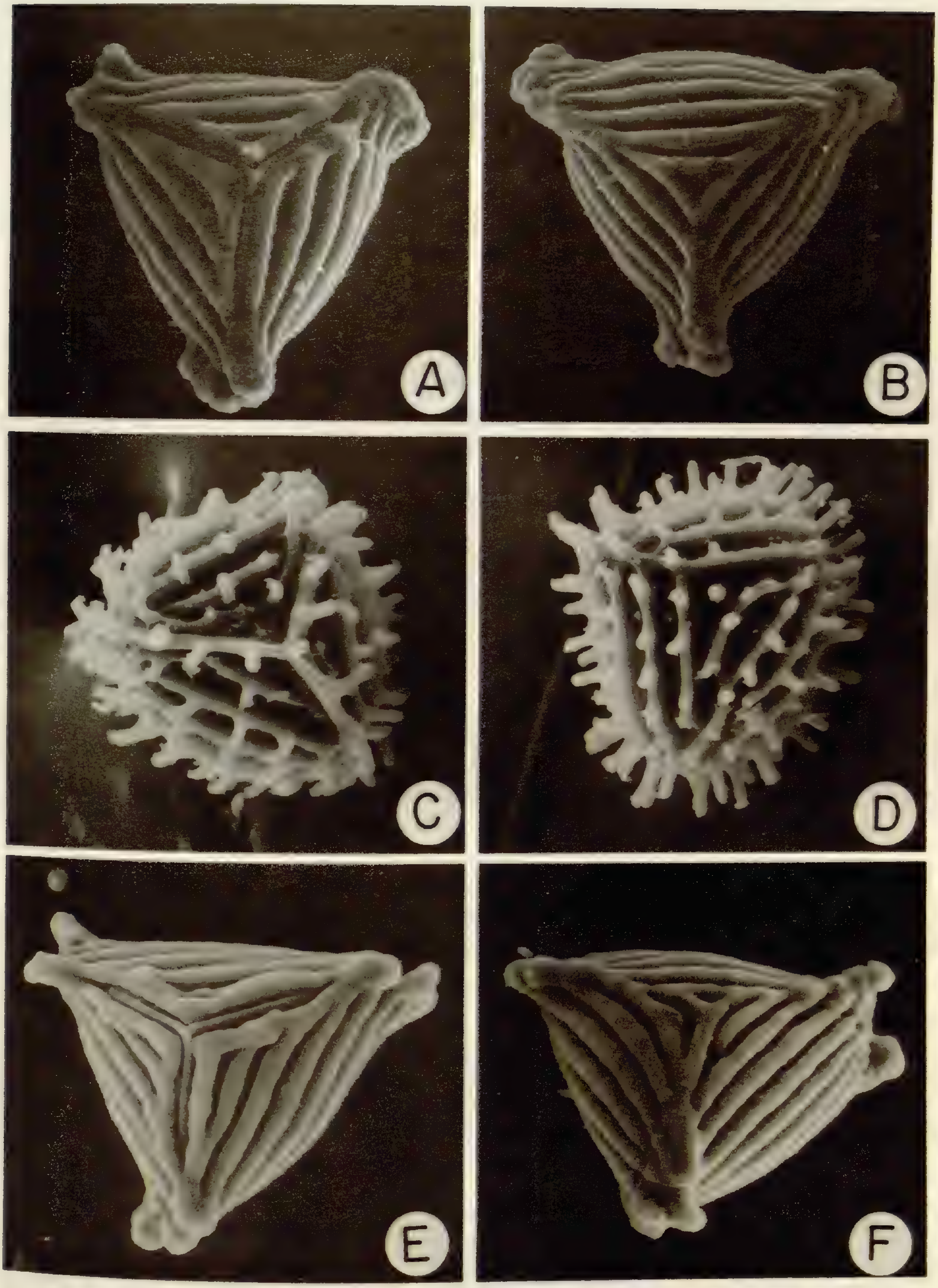


FIG. 2. Spores of *Anemia*. A, B. Proximal and distal faces of *A. antrorsa*, × 467. C, D. Proximal and distal faces of *A. ayacuchensis*, × 600. E, F. Proximal and distal faces of *A. porrecta*, × 467.

Coptophyllum (sect. *Anemibotrys*: *A. aspera*, *A. perrieriana*; sect. *Trochopteris*: *A. elegans*, *A. eximia*), subg. *Anemiorrhiza* (*A. colimensis*), and subg. *Anemia* (sect. *Adetostoma*: *A. brandegeea*, *A. clinata*, *A. intermedia*, *A. salvadorensis*). This is the first report for sect. *Tomentosae* of subg. *Coptophyllum*.

Anemia ayacuchensis Mickel, sp. nov. (Figs. 1A–C).—TYPE: Venezuela, Amazonas, 8 km S of Puerto Ayacucho and just S of the Río Carinagua, savannas between the Río Orinoco and the road, growing at base of boulders, 90 m, 31 Oct 1971, *Davidse 2740* (holotype UC; isotype MO, not seen).

Ab. *A. jaliscana* Maxon sporis spinosis atque rhizomatis pilis cupreis differt.

Rhizome horizontal, compact, 6 mm diam., hairs reddish-orange; fronds erect, sterile fronds 10–16 cm tall, fertile frond 24 cm tall; stipe 0.5 mm diam., ⅓–½ of the sterile frond length, ca. ⅚ of the fertile frond length, stramineous, glabrescent; blade oblong (7 cm long, 2.8–3.6 cm broad), once pinnate, texture papyraceous; pinnae 5–6 pairs, apical pinna obtuse with one (less commonly two) large lateral lobes; pinnae opposite, oblong, truncate to cuneate at base, hirsute on both surfaces, obtuse at apex; margin minutely denticulate; veins free; fertile pinnae erect, long-petiolulate, approximate to the sterile pinnae, far surpassing the sterile blade in height, the ultimate divisions lacking lamina; spores tetrahedral-globose, striate, the ridges narrow with clavate spines, 65–70 (av. 67.8) μm diam. (Figs. 6, 7).

This is amazingly similar to *A. jaliscana* Maxon of western Mexico (see Mickel, 1982), but is distinct in its spore ridges being spiny (rather than smooth) and the rhizome hairs reddish orange (rather than brownish yellow).

Anemia porrecta Mickel, sp. nov. (Figs. 1D–F).—TYPE: Venezuela, Amazonas, along road from Puerto Ayacucho to Samariapo near crossing with Río Cataniapa, savanna and granite dome, on wet granite rock in dense shade of other rock, 31 July 1967, *Wessels Boer 1922* (holotype NY).

Lamina deltata, segmentis rotundatis vel ovatis, et pinnis fertilibus angulo 45° rigide adscendentibus insignis. (L., *porrectus*, stretched out in a straight line, alluding to the habit of the fertile pinnae.)

Rhizome horizontal, compact, 5–6 mm diam., hairs brownish orange; fronds erect, sterile and fertile fronds alike, 9–21 cm tall; stipe 0.6–0.8 mm diam., ca. ½ of the frond length, stramineous, hirsute; blade deltate, 5–10 cm long, 3.3–8.0 cm broad, pinnate to bipinnate-pinnatifid, texture papyraceous; pinnae 5–7 pairs, slightly ascending, apex pinnatifid, pinnae opposite to subopposite, short-petiolulate (1–2 mm), anadromous, 4–6 pairs of pinnules, pinnules and their lobes somewhat rounded or ovate, acute to obtuse, margin shallowly crenulate, hirsute on both surfaces, most densely so on lower surface of costules; veins free; fertile pinnae held rigidly at 45° angle, short-petiolulate (3–5 mm), remote from the sterile pinnae, nearly as long as the longest (basal) sterile pinnae, the ultimate divisions with narrow lamina; spores tetrahedral, vertically compressed, striate,

the ridges broad and smooth with narrow grooves between, 79–99 (av. 89.9) μm diam. (Figs. 2E, F).

Anemia porrecta apparently has no close relatives and probably belongs to subg. *Coptophyllum* sect. *Tomentosae*. The stomata are attached, not floating. The epidermal cells are highly contorted, much like those of *A. elegans* of sect. *Trochopteris*, but the habit is quite unlike members of that group. It is readily identified by its rounded segments and rigidly held fertile pinnae at a 45° angle.

This study was supported in part by a grant from the National Science Foundation, DEB 82-09956. I am indebted to Donald Black for the SEM photomicrographs and Bobbi Angel for the drawings.

Literature Cited

Mickel, J. T. 1982. The Mexican species of *Anemia* (Schizaeaceae). Brittonia 34:388–413.

Review

"*Arkansas ferns and fern allies*," by W. Carl Taylor. 1984. 262 pp. Milwaukee Public Museum. ISBN 0-89326-097-5. $29.95.

This excellent treatment stands, along with R. H. Mohlenbrock's "The Illustrated Flora of Illinois: Ferns" and T. M. C. Taylor's "Pacific Northwest Ferns and Their Allies," among our most beautiful and useful pteridophyte floras. The introductory material on fern morphology, life history, names, and Arkansas geology and fern distribution makes the book entirely comprehensible to neophytes to fern study. The key to genera is thoughtfully illustrated with an example of each genus, and so it is virtually impossible to go wrong at this critical point in making an identification. Seventy-two species in 31 genera are treated. Each species has a short description, brief list of synonyms, statement of habitat and range, and useful notes. The notes include cytological and hybridization information and, in the case of rare or newly discovered species, some information about the plants' discovery. Distribution maps (one dot per county) are included for each taxon, as are very brief specimen lists that provide documentation for the maps. The illustrations, by Paul W. Nelson, are among the best published for United States pteridophytes. Many seem to have been drawn from life. All are reproduced clearly and in large size. The book concludes with an ample glossary, literature cited, a checklist of Arkansas pteridophytes, and an index to common and scientific names. The book is available from the Publications Division, Milwaukee Public Museum, 800 W. Wells St., Milwaukee, WI 53233. Everyone interested in the pteridophytes of the south-central United States or who enjoys excellent illustrations of pteridophytes will want to own a copy.—David B. Lellinger, Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560.

American Fern Journal 75(2):38–43 (1985)

Sporopollenin Content of the Spore Apparatus of *Azolla*[1]

R. E. Toia, Jr., B. H. Marsh, S. K. Perkins, J. W. McDonald, and G. A. Peters

Battelle–C. F. Kettering Research Laboratory, 150 East South College Street, Yellow Springs, Ohio 45387

In 1928 Zetsche and Huggler used the term sporopollenin to describe the exine (outer coat material) obtained from either spores or pollen grains (Shaw, 1971). This material, which is comprised of oxidative, cross-linked polymers of carotenoids and/or carotenoid esters (Brooks & Shaw, 1968; Gooday et al., 1974), is resistant to ultraviolet irradiation, desiccation, biological decay, and non-oxidative chemical attack (Shaw, 1971; Strohl et al., 1977). The extensive work of Zetsche and co-workers (1928–1937), and the later studies of Shaw and co-workers (1964–1974), summarized by Brooks and Shaw (1971) and Shaw (1971), have provided substantial information on the chemistry, biochemistry, and geochemistry of sporopollenin.

Sporopollenin has a wide taxonomic distribution, occurring in myxobacteria, fungi, algae, pteridosperms, gymnosperms, and angiosperms (Brooks, 1971; Atkinson et al., 1972; Gooday et al., 1974; Strohl et al., 1977; Honegger & Brunner, 1981). Sporopollenin in sporocarps of *Azolla*—a heterosporous, free-floating fern that occurs worldwide in freshwater habitats (Moore, 1969)—was inferred histochemically (Lucas & Duckett, 1980), but not chemically defined. Therefore, we undertook this effort to verify the presence of sporopollenin in *Azolla* spores, and gain some insight into its contribution to the structure of spore apparatus. We have now identified this compound in *Azolla* spore apparatus by infrared spectroscopy (IR) following acetolysis. The percent (by weight) sporopollenin of the spore apparatus has been determined, and its contribution to spore apparatus architecture demonstrated with scanning electron microscopy prior to and following acetolysis.

Materials and Methods

Azolla mexicana Presl was used as the source of sporocarps. The original population was collected from the Graylodge State Waterfowl Area, Butte County, California, during the summer of 1974 by S. Ela. The culture we utilized was obtained from D. W. Rains and co-workers of the University of California–Davis in 1978, and subsequently maintained at this laboratory. Cultures were grown on nitrogen-free IRRI medium (Peters et al., 1980) and maintained under an illumination of 100–200 $\mu E/m^2/s$, with a 16/8 hr, 26/18°C light-dark regime. Voucher specimens are maintained at the New York Botanical Garden. Mature

---

[1] Contribution No. 865 from the Battelle–C.F. Kettering Research Laboratory.

megasporocarps, microsporocarps, and microsporangia were harvested from the frond material by agitation in large volumes of water, and separated from the majority of the frond debris by sieving. Individual spore types were isolated by centrifugation on Percoll discontinuous density gradients at 16,000 × g for 5 min. Microsporangia banded at the 15–30% Percoll interface, microsporocarps banded in the 30% Percoll region, megasporocarps sedimented into the 45% Percoll layer, and the remaining debris formed a pellet on the bottom of the centrifuge tube. The spore types were removed from the gradients and repeatedly washed with water. They were then air dried at room temperature for 48–72 hr, and stored desiccated at room temperature.

The sporopollenin of *Lycopodium* spores has been well characterized by chemical studies (Brooks, 1971; Shaw, 1971) and was employed as a reference material in this study. The *Lycopodium* spores were kindly provided by W. Doyle, University of California–Santa Cruz; the material was originally obtained from Carolina Biological Co. as *Lycopodium* powder, and, as such, may represent a mixture of spores from more than one *Lycopodium* species.

Acetolysis of the dried material was based on the method of Atkinson et al., 1972. The acetolysis residues were ground into a homogenous powder with KBr as a matrix substance, and pelleted at high pressure for use in infrared spectroscopy. Spectra of these samples were recorded on a Beckman IR-20A Infrared Spectrophotometer.

A Perkin-Elmer Model 240 Elemental Analyzer, equipped with an MC-341 Microjector from Control Equipment Corp. for sample automation, was used to analyze dried, pulverized samples of spore material for carbon, hydrogen, and nitrogen.

For scanning electron microscopic (SEM) observations, acetolysis residue material was mounted on aluminum SEM stubs with double-sided tape, and sputter coated with 20 nm platinum. Fresh spore material, which was used for comparison, was fixed in 2% glutaraldehyde in 50 mM PO_4, pH 7.2, dehydrated in a graded acetone series, and critical point dried using liquid CO_2 as the transition fluid. This material was then mounted and coated as indicated above. Examination was performed in an ISI DS–130 scanning electron microscope.

Results and Discussion

Sporopollenin can be defined at the practical level as acetolysis-resistant material (Brooks, 1971; Atkinson, 1972). IR spectra of acetolysis-resistant material obtained from *Azolla* spore apparatus compare very well with those obtained for *Lycopodium* spores carried through the same procedure (Fig. 1), as well as with published spectra for *Lycopodium* sporopollenin (Atkinson et al., 1972; Honegger & Brunner, 1981). The minor differences between the spectra are probably due either to the fact that sporopollenin preparations exhibit variability since their precursors (carotenoids and/or carotenoid esters) vary among organisms (Strohl et al., 1977), or to technical difficulties in grinding the acetolysis-resistant material into a homogenous powder in preparation for IR analysis (Honegger & Brunner, 1981). The band at approximately 2350 cm^{-1} could be due to

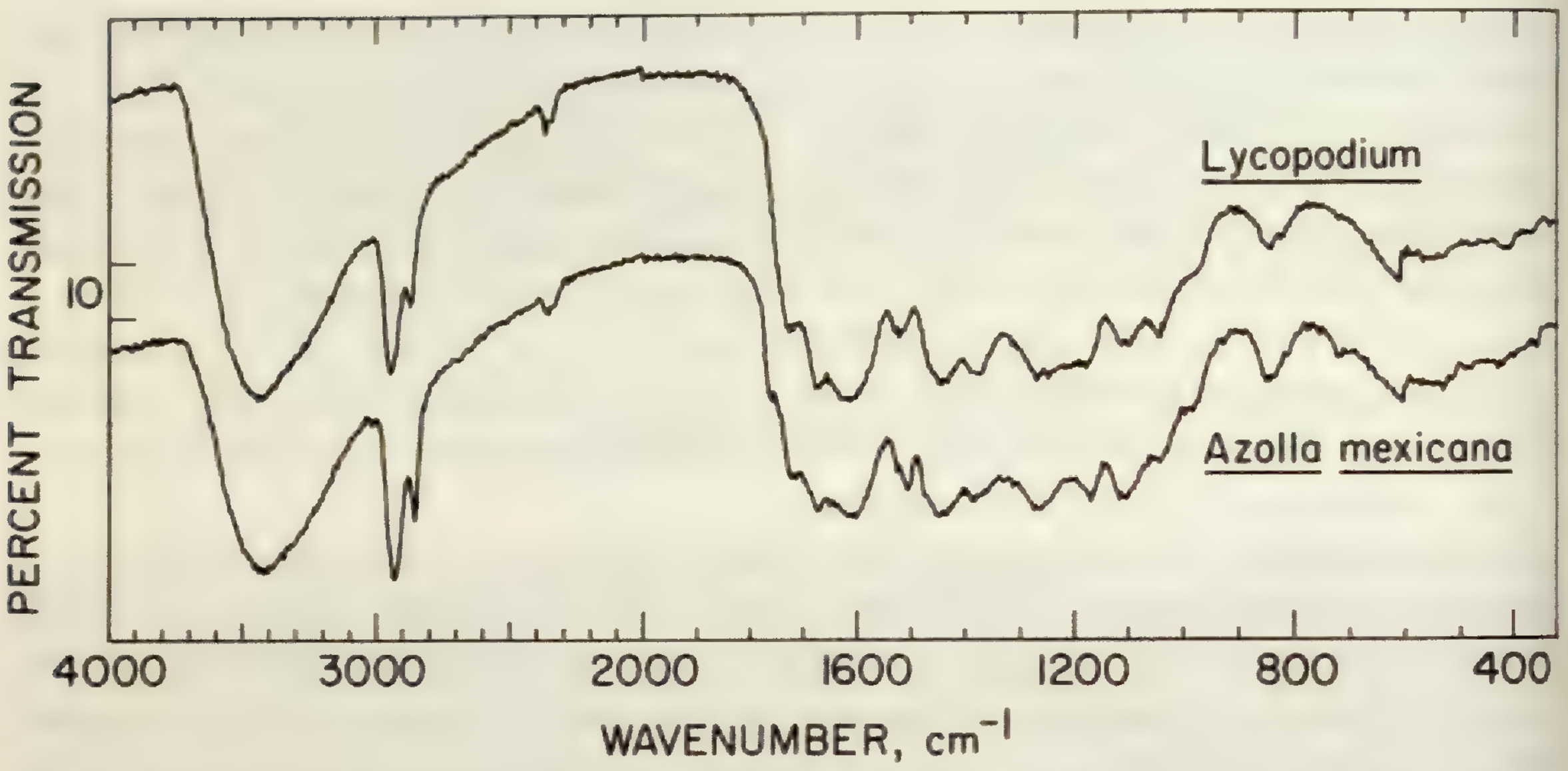


FIG. 1. Infrared absorption spectra of acetolysis residues from *Azolla mexicana* and *Lycopodium*.

the presence of a silicon hydride. This band occurs in spectra reported in the literature, but has not been discussed. An independent laboratory analysis (Galbraith Laboratories, Inc., Knoxville, Tennessee) of our dried *Azolla* sporocarps showed 0.30% silicon on a dry weight basis. Our acetolysis-resistant preparations from both *Azolla* and *Lycopodium* were completely solubilized by 20% chromic acid. This result is consistent with the properties of sporopollenin, as well as with the occurrence of Si as only a very minor component (Atkinson, 1972; Strohl et al., 1977; Good & Chapman, 1978).

Sporopollenin content of *Azolla* megasporocarps, microsporocarps, and microsporangia is higher than that found in *Lycopodium* spores (Table 1). A sporopollenin content of 24% for *Lycopodium* spores agrees exceptionally well with previous reports of 23.4 to 23.8% (Brooks, 1971; Brooks & Shaw, 1971). The highest previously reported value of 31.8% for *Selaginella kraussina* spores (Brooks, 1971) is approximated by the value we obtained for sporopollenin content of *Azolla* microsporangia (30%), while it is exceeded by the values obtained for megasporocarps (43%) and microsporocarps (45%).

The carbon, hydrogen, and nitrogen content of *Azolla* spore apparatus and *Lycopodium* spores was determined before and after acetolysis (Table 2). Following acetolysis the preparations were essentially N-free, consistent with the

TABLE 1. Sporopollenin Content of Various *Azolla* Spore Apparatus (and *Lycopodium* Spores as a Standard).

Sample	% Sporopollenin
A. mexicana megasporocarps	43
A. mexicana microsporocarps	45
A. mexicana microsporangia	30
Lycopodium spores	24

TABLE 2. CHN Content of Various *Azolla* Spore Apparatus (and *Lycopodium* Spores as a Standard) Before (a) and After (b) Acetolysis.

Sample	% C		% H		% N	
	a	b	a	b	a	b
A. mexicana megasporocarps	59.1 ± 1.0	60.3 ± 1.2	8.4 ± 0.9	5.5 ± 0.0	3.7 ± 0.3	0.1 ± 0.0
A. mexicana microsporangia	58.4 ± 0.7	58.0 ± 0.4	8.2 ± 0.4	5.2 ± 0.0	1.0 ± 0.2	0.0 ± 0.0
Lycopodium spores	67.5 ± 0.4	63.1 ± 0.2	9.9 ± 0.1	5.4 ± 0.0	1.3 ± 0.0	0.0 ± 0.0

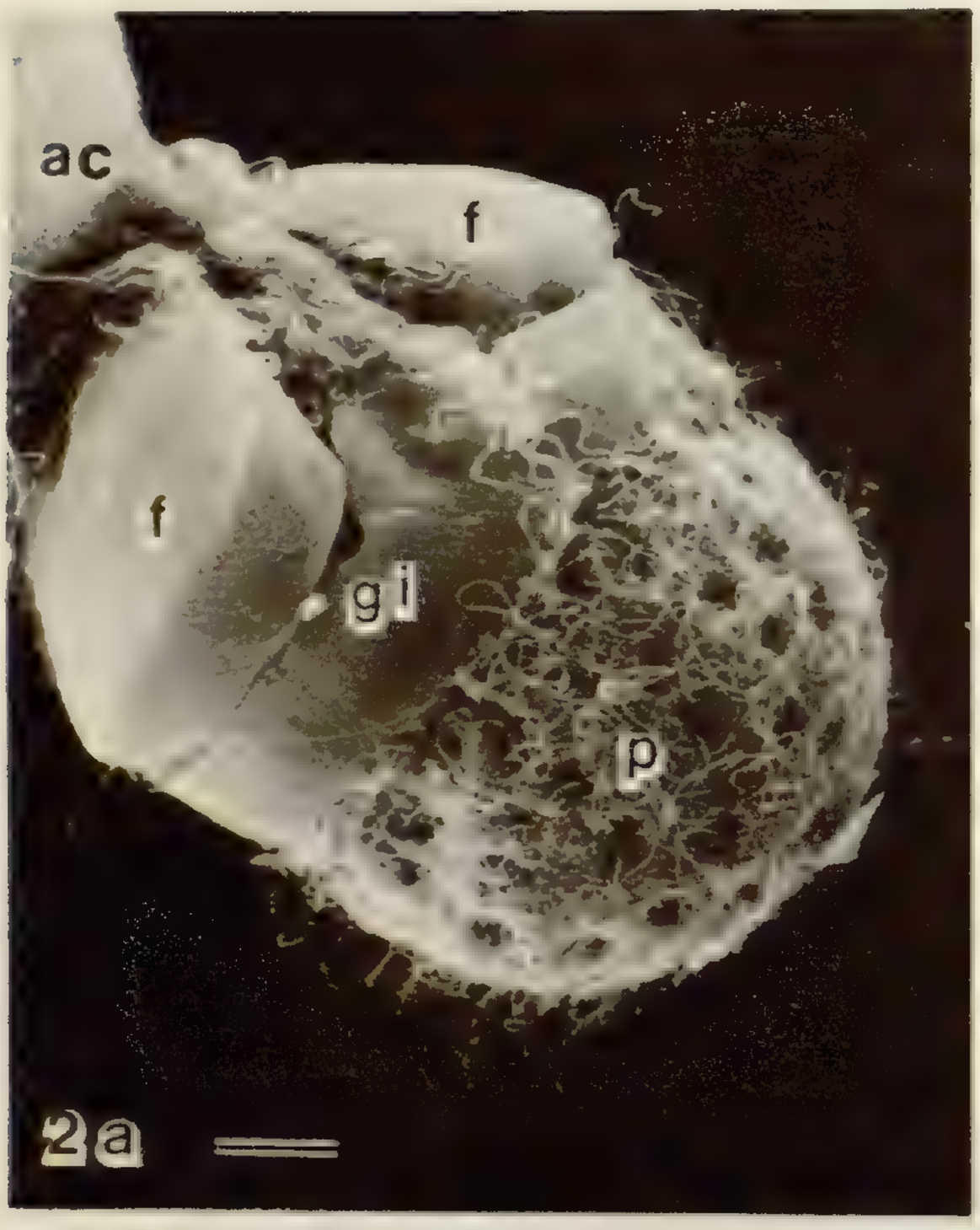
composition of all known sporopollenins (Brooks & Shaw, 1968, 1971; Shaw, 1971). From this analysis we postulate that *Azolla* sporopollenin has an empirical formula of $C_{90}H_{132}O_{33}$ (arbitrarily expressed in terms of C_{90} units, cf. Brooks & Shaw, 1971; Shaw, 1971), assuming that only carbon, hydrogen and oxygen are present. This formula is well within the range of published values for sporopollenins from different sources (Shaw, 1971).

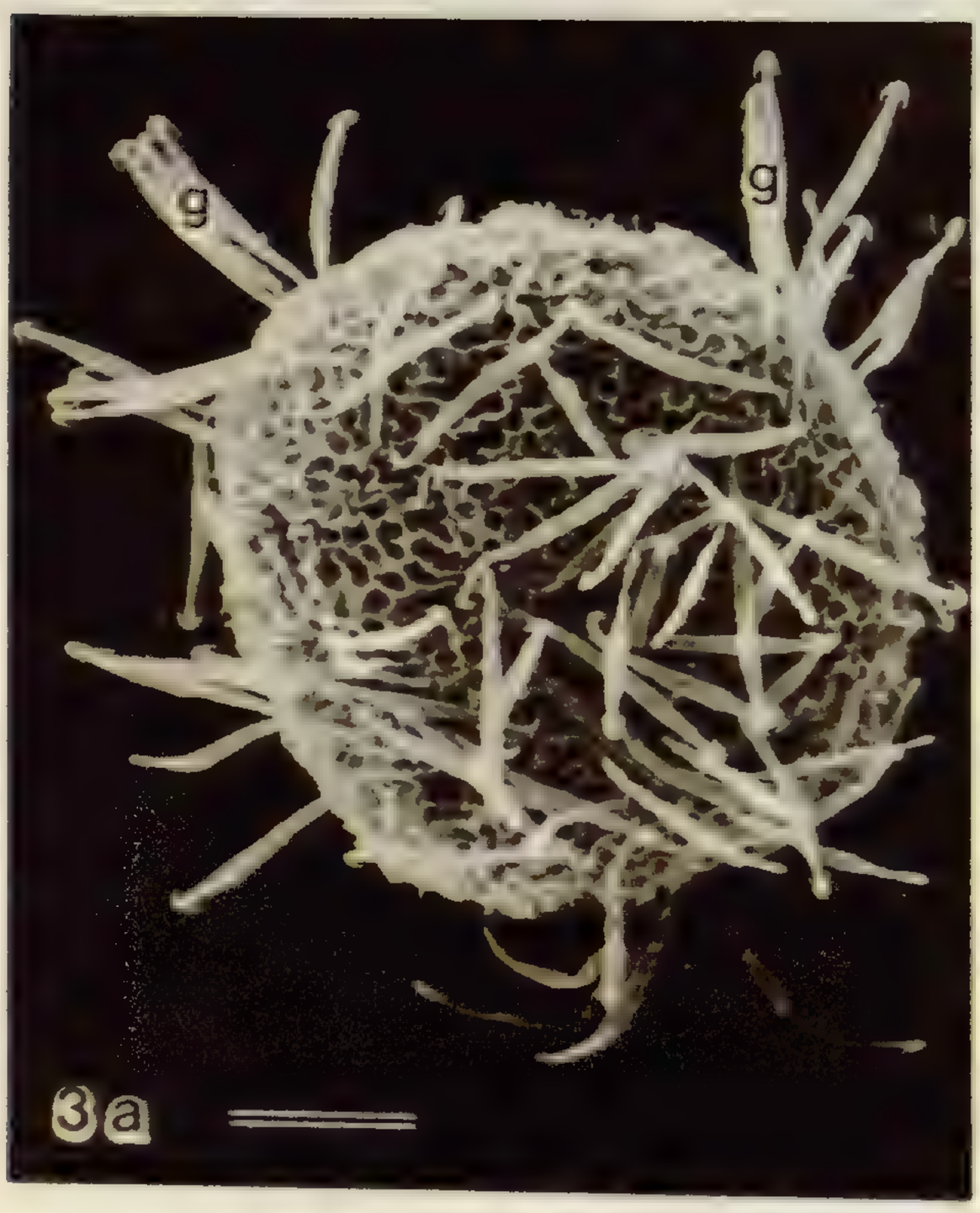
Having conclusively demonstrated that the acetolysis-resistant component of the *Azolla* spore apparatus is sporopollenin, the effect of acetolysis on morphology was assessed with SEM. In general, the overall morphological characteristics are remarkably similar before and after treatment. This implies that the structures have a large sporopollenin content, thus corroborating our chemical analysis.

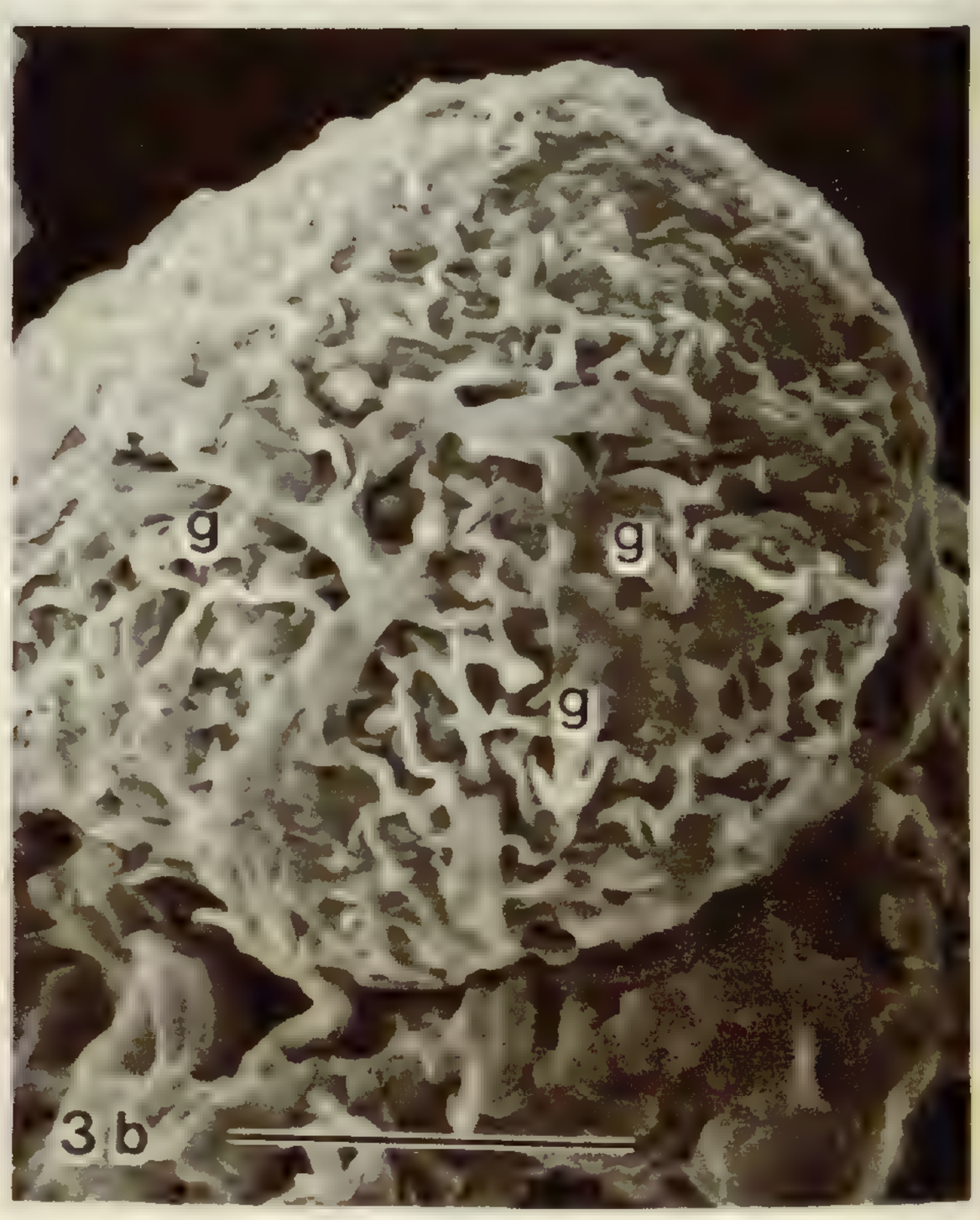
A megasporocarp of *A. mexicana* with the indusium removed (Fig. 2a) reveals four external structures: the perine covering the megaspore, the girdle above the perine, the floats which are recessed into the girdle, and the apical cap sitting atop the floats. The surface of the perine is composed of an entwined labyrinth of filamentous processes with localized areas devoid of these excrescences, giving the perine a dimpled appearance. Long filamentous hairs extend over the surface of the perine and comprise the capture mechanism (Calvert et al., 1983). SEM observations following acetolysis (Fig. 2b) showed the megasporocarps to be somewhat shrunken; however, the gross morphology was unchanged. The fine structure of the perine appeared less distinct, with the filamentous hairs of the capture mechanism fused to the perine surface. The girdle and floats appeared collapsed and contracted, with the floats deeply recessed into the girdle. Dissection of the surface of the floats (Fig. 2b) revealed that the internal alveolate organization was not disrupted by acetolysis.

Microsporocarps contain microsporangia which in turn contain massulae. The massulae are pseudocellular, alveolate masses thought to be composed almost entirely of sporopollenin (Lucas & Duckett, 1980). The surface of each massula bears elongate processes termed glochidia. As with the megasporocarp, the gross morphology of the massulae was quite similar before (Fig. 3a) and after (Fig. 3b) acetolysis. However, while Lucas and Duckett (1980) indicated that the massulae and glochidia were strongly resistant to acetolysis, our results suggest a moderate susceptibility. Following acetolysis the alveolate nature of the massular surface was less distinct and, while discernible, the glochidia had collapsed onto the surface of the massula.

These studies do not allow us to state whether any specific structure contains







FIGS. 2 and 3. Scanning electron micrographs of *Azolla mexicana* spore apparatus. Scale bars = 50 μm. 2, Megasporocarp before (a) and after (b) acetolysis, showing the perine (p), girdle (gi), floats (f), and apical cap (ac). 3, Intact massula from microsporocarp, before (a) and after (b) acetolysis, showing glochidia (g).

only sporopollenin, or for that matter, no sporopollenin. However, they have documented the presence of sporopollenin by IR spectroscopy, and have clearly shown that it is a major structural component of *Azolla* sporocarps, with levels of sporopollenin significantly exceeding those reported previously.

The authors are indebted to S. R. Dunbar and M. Z. Tootle for assistance in figure preparation. The micrograph in Figure 3a was kindly provided by H. E. Calvert of this laboratory. This work was supported in part by National Science Foundation Grant PCM-820845802 awarded to G.A.P.

Literature Cited

Atkinson, Jr., A. W., B. E. S. Gunning, and P. C. L. John. 1972. Sporopollenin in the cell wall of *Chlorella* and other algae: Ultrastructure, chemistry, and incorporation of ^{14}C-acetate, studied in synchronous cultures. Planta 107:1–32.

Brooks, J. 1971. Some chemical and geochemical studies on sporopollenin. Pp. 351–407 in *Sporopollenin*, ed. J. Brooks et al. New York: Academic Press.

Brooks, J. and G. Shaw. 1968. Chemical structure of the exine of pollen walls and a new function for carotenoids in nature. Nature 219:532–533.

——— and ———. 1971. Recent developments in the chemistry, biochemistry, geochemistry and post-tetrad ontogeny of sporopollenins derived from pollen and spore exines. Pp. 99–114 in *Pollen: Development and physiology*, ed. J. Heslop-Harrison. London: Butterworths.

Calvert, H. E., S. K. Perkins, and G. A. Peters. 1983. Sporocarp structure in the heterosporous water fern *Azolla mexicana* Presl. Scanning Electron Microscopy III:1499–1510.

Good, B. H. and R. L. Chapman. 1978. The ultrastructure of *Phycopeltis* (Chroolepidaceae: Chlorophyta). I. Sporopollenin in the cell walls. Amer. J. Bot. 65:27–33.

Gooday, G. W., D. Green, P. Fawcett, and G. Shaw. 1974. Sporopollenin formation in the ascospore wall of *Neurospora crassa*. Arch. Microbiol. 101:145–151.

Honegger, R. and U. Brunner. 1981. Sporopollenin in the cell walls of *Coccomyxa* and *Myrmecia* phycobionts of various lichens: an ultrastructural and chemical investigation. Canad. J. Bot. 59:2713–2734.

Lucas, R. C. and J. G. Duckett. 1980. A cytological study of the male and female sporocarps of the heterosporous fern *Azolla filiculoides* Lam. New Phytol. 85:409–418.

Moore, A. W. 1969. *Azolla*: Biology and agronomic significance. Bot. Rev. 35:17–34.

Peters, G. A., R. E. Toia, Jr., W. R. Evans, D. K. Crist, B. C. Mayne, and R. E. Poole. 1980. Characterization and comparisons of five N_2-fixing *Azolla—Anabaena* associations. I. Optimization of growth conditions for biomass increase and N content in a controlled environment. Plant, Cell and Environ. 3:261–269.

Shaw, G. 1971. The chemistry of sporopollenin. Pp. 305–350 in *Sporopollenin*, ed. J. Brooks et al. New York: Academic Press.

Strohl, W. R., J. M. Larkin, B. H. Good, and R. L. Chapman. 1977. Isolation of sporopollenin from four myxobacteria. Canad. J. Microbiol. 23:1080–1083.

American Fern Journal 75(2):44–55 (1985)

Role of Morphological Characteristics of Leaves and the Sporangial Region in the Taxonomy of *Isoetes* in Northeastern North America

L. S. Kott[1] and D. M. Britton
Department of Botany and Genetics, University of Guelph, Guelph, Ontario

Vegetative features have often been used as key characters in distinguishing between species of *Isoetes* as well as for supportive evidence in taxonomic treatments of the genus (Pfeiffer, 1922; Fernald, 1950). As part of a larger taxonomic study of a group of species within this genus from northeastern North America, morphological characteristics of the vegetative body were studied in order to assess their taxonomic significance. Taxonomically valuable character states were considered to be those that correlated with distinctive spore ornamentation (Kott & Britton, 1983), cytological (Kott & Britton, 1980), chemical (Kott & Britton, 1982a) and biological groupings (Kott & Britton, 1982b). Those characters that varied within taxa and overlapped greatly among taxa were considered of no taxonomic value. Characters of the leaf, corm, roots, velum, ligule, leaf margins, and sporangium were investigated.

Materials and Methods

Morphometric studies were made from specimens borrowed from herbaria (CU, DAO, GH, LKHD, MICH, MIN, MO, NEBC, NY, OAC, PFES, QFA, QUE, SFS, TRT, UVIC, VT, WAT, and WIS). Additional specimens were received from Algonquin Park Museum (APM), and the personal collection of B. Gauthier. The authors' collections have been deposited at the University of Guelph (OAC). The species of *Isoetes* considered in this study were *I. macrospora* Dur., *I. hieroglyphica* A. A. Eaton, *I. riparia* Engelm. in A. Braun, *I. tuckermanii* A. Braun, *I. acadiensis* Kott, *I. echinospora* Dur., *I. eatonii* Dodge, and *I. engelmannii* A. Braun, all from northeastern North America.

Leaf measurements were made to the nearest half centimeter with a plastic millimeter rule, which could be bent to conform to the curvature of the leaves. Measurements were made from the upper surface of the corm at the base of the leaf to leaf tip; only the longest leaf per plant was measured. On most plants, leaves of one age class were generally similar in height, the younger and shorter ones being nearer the center of the corm at the apex. Leaf margin measurements were made on the oldest complete leaf.

Leaf number was determined by counting all leaves per plant measuring over 1 cm including old, outer leaves, often represented only by persistent leaf bases.

---

[1] Present address: Department of Crop Science, University of Guelph, Guelph, Ontario.

Leaf number and length were determined on more than 3000 plants in about 600 populations.

Variation in leaf number and length was calculated for plants within a few large but typical populations and between all populations available of each species. The statistic used for comparisons of the variability of leaf features was the coefficient of variability:

$$CV = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

Leaf habit (relating to the presence or absence of curvature of individual leaves) was scored as follows: 1) erect, leaves upright and stiff; 2) slightly recurved, leaves essentially erect but slightly reflexed at the tips; and 3) recurved, leaves decidedly recurved or reflexed in a fixed position.

Leaf texture (relating to thickness of individual leaves) was scored only when the leaves were 1) of unusual thinness or appeared fine and soft, or 2) unusually thick or stout, almost brittle. Leaves that had neither of these characters were considered of "typical" texture and were not scored.

Leaves were scored for color when they were unusually dark green, yellow-green, or red-green, but the color of most dried specimens had deteriorated or faded and accurate data were impossible to accumulate for all specimens. The more recently collected specimens gave better color data. Stomata were observed by the acetate peel method. Data for leaf habit, texture, and color were scored for 466 populations.

Height and width of mature sporangia were measured in mm at the longest dimension. Velum length was measured from the base of the ligule down to the free edge of the velum towards the center of the sporangium. Ligules of mature leaves were observed, measured, and drawn from live specimens only. Sporangium coloration was observed and photographed from slides of dry sporangial walls mounted in Euparol, or observed directly with a stereoscope. Sporangium coloration was scored as unspotted (clear), spotted, patchy, or tan.

Results and Discussion

General Morphology.—The most obvious feature of an *Isoetes* plant is the tuft of green, linear leaves, congested at their bases, borne on a solid corm in a centrifugal, spiral pattern. The corm is a short stem-like organ that may be variously lobed. Some species such as *I. japonica* A. Braun of Japan, *I. durieui* Bory of southern Europe, and *I. coromandelina* L.f. of India (Pfeiffer, 1922) have three-lobed corms. Plants of northeastern North America generally have a two-lobed corm, and therefore this feature has no discriminatory value.

Lobes of the corm are separated by shallow fossae. Young roots develop from the basal meristem in the lower part of the corm along this fossa (Paolillo, 1963). The flattish shoot apex sits in a central concavity on top of the corm. Older leaves are displaced from the shoot apex by development of younger leaves. Therefore, the oldest leaves and roots are found at the periphery of the corm where old leaves, roots, and deteriorated sections of old corm tissues are sloughed. The age

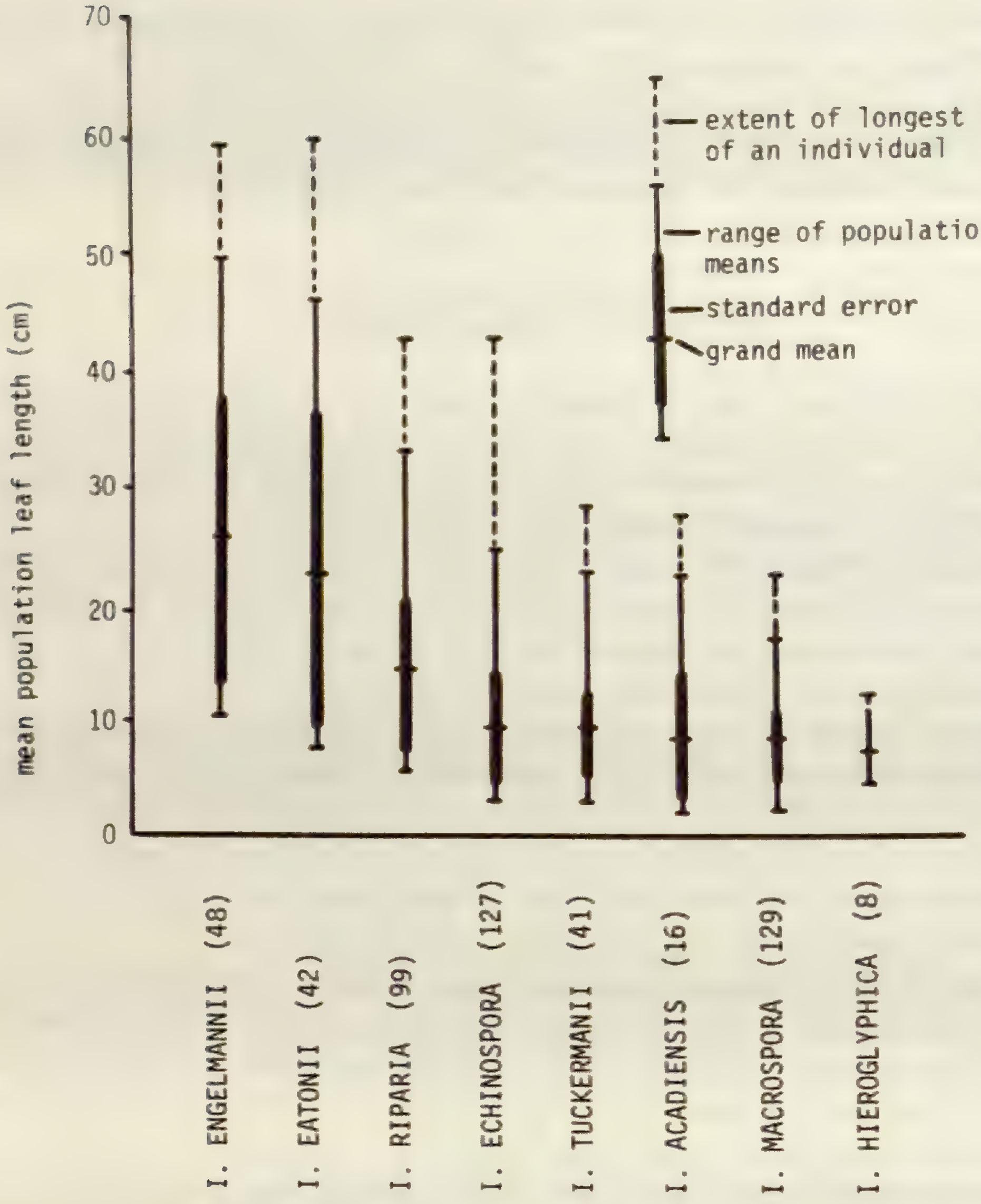


FIG. 1. Comparison of leaf length of *Isoetes* based on population means. Number of populations sampled indicated parenthetically for each taxon.

of a corm is therefore virtually impossible to determine with accuracy. Karrfalt (1977) speculated on the age of *Isoetes* corms; however, his studies did not consider tissue previously shed, and therefore his results cannot be conclusive.

Roots emerging from the base of the corm have a large root cap (Peterson et al., 1979). The roots branch dichotomously and have a stigmarian type of anatomy, possessing a large central air cavity surrounded by cortex, with the vascular tissue eccentrically placed. While roots are young and actively growing, they appear white. Later, only the root tip remains white, while the body of the root becomes a uniform brown. The gross morphology of roots does not provide characteristics of taxonomic value because the roots are morphologically very constant and generally behave in the same manner in all species considered in this study.

The leaves provide all of the taxonomically important vegetative information

TABLE 1. Variability of Leaf Length and Number within Sample Populations of *Isoetes* spp.[1]

	Leaf length			Leaf number		
	Mean (cm)	SD	CV	Mean	SD	CV
I. macrospora	5.6	1.3	22.2	11.6	3.1	26.4
	6.8	2.0	28.7	21.7	4.0	18.6
I. tuckermanii	8.9	2.6	28.7	11.1	3.0	26.7
	9.1	1.7	18.5	13.4	3.1	22.7
I. riparia	14.8	2.8	19.1	11.0	3.7	34.0
	10.5	1.3	12.6	11.3	4.2	37.5
I. acadiensis	22.2	3.0	13.5	17.8	8.8	49.8
	9.1	2.7	29.7	28.0	12.6	45.0
I. echinospora	9.6	3.1	32.0	15.5	4.3	29.9
	7.6	1.5	20.0	14.2	2.9	20.2
I. eatonii	14.5	2.7	18.9	34.0	7.2	21.1
	47.4	7.5	15.8	46.0	49.8	108.3
I. engelmannii	11.4	1.8	15.6	21.4	2.7	12.6
	18.8	1.3	6.9	16.0	9.2	57.6

[1] *Isoetes hieroglyphica* plants were too few to analyze statistically.

about the species. Leaves are awl-shaped, but vary from very fine and thread-like throughout to thick and stout, flaring at the base and with an abrupt point at the tip. Leaf color is typically bright green, but varies from yellow-green, to dark green, and occasionally to reddish-green. Those parts of the leaves that are buried in the substrate are opaque white or brown. Leaf bases of some species that occur outside the study area are chestnut-brown and persistent as in *I. melanopoda* Gay & Dur.

Internally the leaves have four longitudinal air chambers that are traversed by thin diaphragms at regular intervals. The vascular tissue is located in the central tissue of the leaf.

Leaf Characteristics.—Leaf measurements have been used as supporting diagnostic evidence in several keys (Eaton, 1900, 1908; Pfeiffer, 1922) but data presented here indicate that leaf length, at least in part, is variable and perhaps strongly dependent on ecological conditions and the vigor of the plant. Measurements of leaf length yielded results seen in Figure 1. The standard error is large for taxa that have large leaf length ranges reflecting the great sensitivity of the leaf length to environmental conditions.

Isoetes engelmannii and *I. eatonii* had the longest leaves, with means of 26 and 23 cm respectively, but both also had considerable range of variability as shown by the high standard errors. *Isoetes riparia* had a grand mean of 15 cm with population means of 33 cm at the upper end of its range. The remaining species had leaf length grand means between 8 and 10 cm. Even the leaf length ranges are similar for these last species. This observation indicates that leaf length is not a good taxonomic character for most of these species.

The variability of leaf length within some sample populations is shown in Table 1 as coefficients of variability (CV). A high CV value indicates a high

TABLE 2. Variability of Leaf Length and Number between Populations within Species of *Isoetes*.

	Leaf length				Leaf number			
	Grand mean (cm)	Range of $\bar{x}$	SE	CV	Grand mean (cm)	Range of $\bar{x}$	SE	CV
I. macrospora	7.6	3–17	2.7	34.4	19.1	4–53	11.6	60.6
I. hieroglyphica[1]	8.0	5–11	—	—	13.0	7–16	—	—
I. tuckermanii	9.2	4–23	3.6	39.1	22.4	10–45	8.5	37.9
I. riparia	14.6	6–33	6.2	42.5	14.8	5–36	7.0	47.3
I. acadiensis	9.3	2–21	5.0	53.9	25.6	9–35	15.2	59.6
I. echinospora	9.6	4–25	4.4	46.2	14.5	7–26	4.4	30.4
I. eatonii	22.7	8–46	13.5	59.3	46.6	12–98	31.2	67.1
I. engelmannii	26.0	10–50	11.8	45.6	27.2	9–61	17.1	63.1

[1] *Isoetes hieroglyphica* populations too few to analyze statistically.

degree of variability, whereas a low CV value indicates a low degree of variability. All CV values for leaf length were above 6.9, generally most were over 18, and the highest being 32 (Table 1).

Between populations, leaf length CV values were even higher than within populations. These high values, which ranged from 34 to 59 (Table 2), reflect the effect of environment on individual plants during the time of leaf development.

Leaf number per plant was recorded because this number has been used as diagnostic or supportive taxonomic evidence in the past. Results of the means and the ranges of leaf number per plant are shown in Figure 2. *Isoetes eatonii* had the highest grand mean at 47 leaves per plant, as well as the largest range of population means (12–98). *Isoetes acadiensis*, *I. tuckermanii* and *I. engelmannii* had grand means in the 20s, while the remaining species had grand means below 20 leaves per plant. The extremely large variation in leaf number (=range) within species strongly suggests that leaf number is a function of factors such as age and vigor of plants. Habitat perhaps may play an important role since plants inhabiting shallow waters may have a reduced leaf number due to wave or ice action, while those in deep, undisturbed waters may exhibit higher leaf numbers since older leaves can accumulate on the corm.

Variability in leaf number was compared among plants of a few sample populations and among populations within a species. The CV values of leaf number within a population are again very high and are generally considerably higher than comparable CV values of leaf lengths (Table 1). They range from 12 to 108, half being 30 or higher. Older plants tend to have more leaves because they have a larger corm, while it takes new plants several years to build up to an average number of leaves. Because a population consists of both young and old individuals, leaf number is not reliable diagnostic feature.

The CV values also tend to be higher within species (i.e., between populations) than within populations (Table 2). They range from 30 to 67. Such values show great variability of leaf number from plant to plant and from population to population.

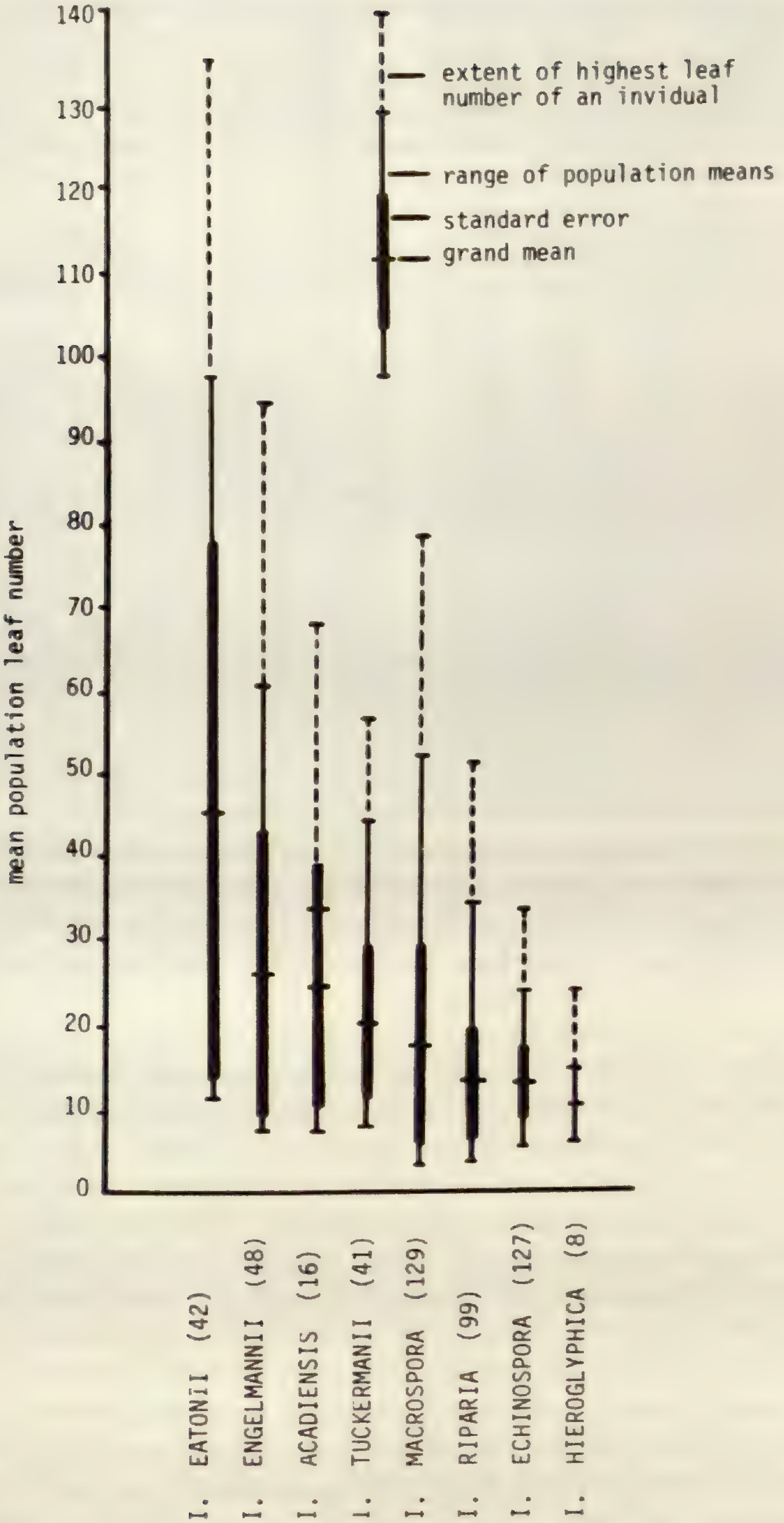


FIG. 2. Comparison of leaf number of *Isoetes* based on population means. Number of populations sampled indicated parenthetically for each taxon.

TABLE 3. Tendencies in Gross Leaf Characteristics in *Isoetes* spp.[1] (Expressed as a Percentage).

	Leaf habit %				Leaf texture %[2]			Leaf color %[3]			
	Erect	Slightly recurved	Recurved	Total pops.	Soft and slender	Stiff and stout	No. pops.	Yellow-green	Dark green	Red-green	No. pops.
I. macrospora	39	27	34	106	35	65	26	6	91	3	33
I. tuckermanii	63	19	18	77	91	9	56	71	14	14	14
I. riparia	100			87	95	5	42	79	21		14
I. acadiensis	25	25	50	12	100		12		75	25	4
I. echinospora	73	14	14	103	83	17	30	43	29	29	8
I. eatonii	100			40	92	9	26	100			10
I. engelmannii	98	2		41	80	20	15	100			7

[1] *Isoetes hieroglyphica* had too few specimens for reliable data.
[2] Includes only those plants that do not fall into "typical" texture for genus.
[3] Includes only those plants that were not "typical" bright green.

Isoetes eatonii generally had erect leaves with a soft and lax leaf texture, yet a few specimens were erect with very stout leaves. *Isoetes tuckermanii* exhibited erect, fine leaves, but many populations also had leaves either slightly or severely recurved. *Isoetes riparia* had erect or straight leaves that often appeared to have been trained by the water current. *Isoetes macrospora* and *I. acadiensis* had a mixture of all three leaf types but exhibited the recurved habit half of the time. While *I. acadiensis* leaves were primarily fine-textured, those of *I. macrospora* were most often stout and rigid. The common state for *Isoetes echinospora* leaves was erect, but leaves were occasionally slightly recurved or reflexed. There was a tendency for the leaves to be fine. *Isoetes engelmannii* leaves were also generally erect and usually somewhat lax.

Qualitative characters such as leaf habit and texture are often quite arbitrary and do not easily conform to classes. An effort was made to score leaf form as a combination of leaf habit and leaf texture. Table 3 is not intended to show the actual numerical representation of leaf form for each taxon, but instead, to show prevailing trends in each taxon. "Leaf texture" (Table 3) includes only those populations in each species that did not fall into the "typical" texture state, and is a score of those plants with leaf characters tending either toward fine and soft or thick and stout. These atypical populations included about one third of those scored.

Of approximately 600 populations scored for leaf color, only 90 were atypical and fell into categories of yellow-green, dark green and red-green. The typical color was bright green. *Isoetes eatonii* was yellow-green or bright green, *I. tuckermanii* mostly yellow-green or green with some tending toward reddish-green. *Isoetes riparia* ranged from a high number of yellow-green and bright green populations to some that were dark green. *Isoetes acadiensis* and *I. macrospora* both tended towards the dark green leaf color, occasionally being reddish-green. The color of *I. echinospora* and *I. engelmannii* leaves were typically bright green, but both tended towards yellow-green (Table 3).

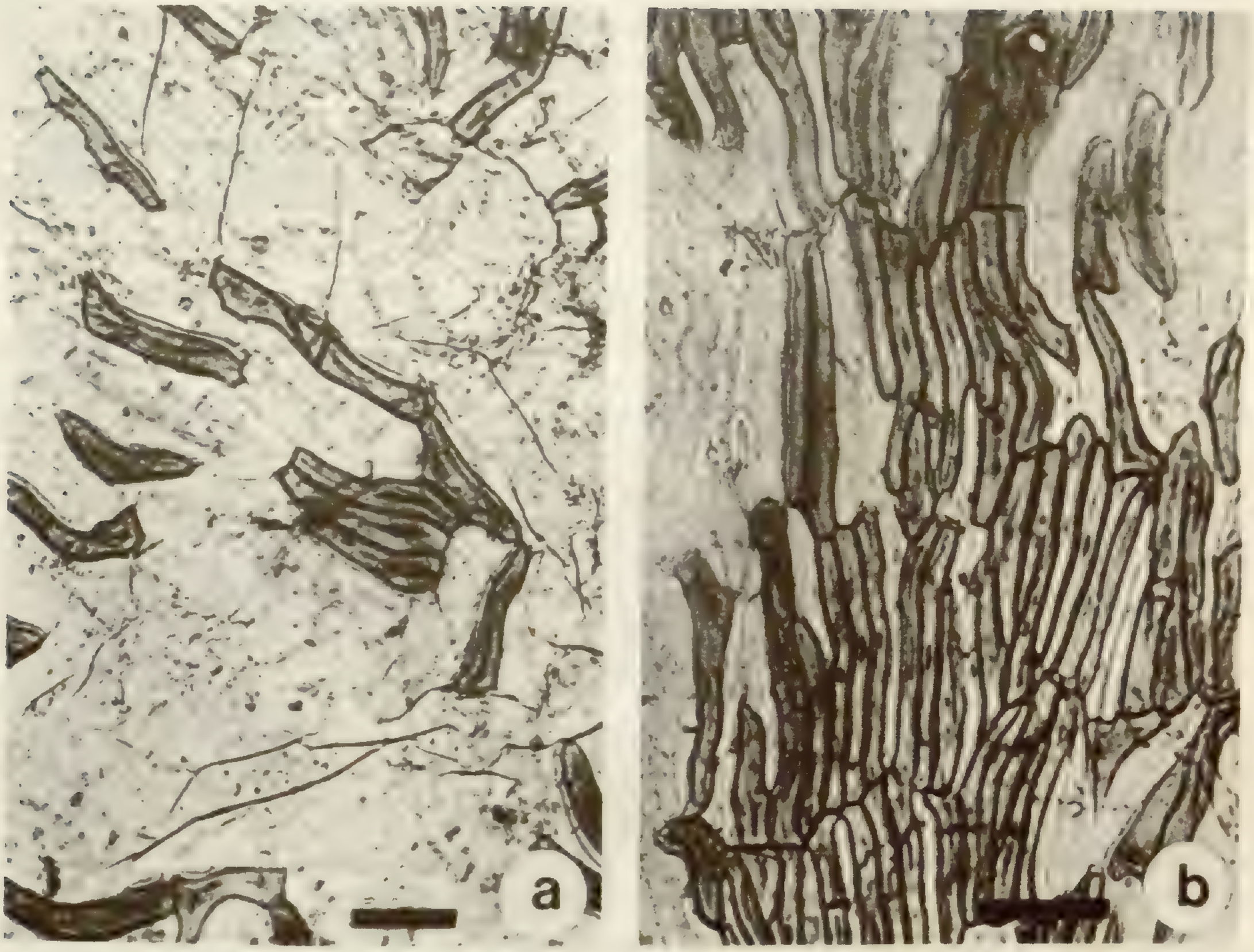


FIG. 3. Coloration of sporangial walls. Bar = 50 μm. a, Pale brown scattered cells of *Isoetes riparia* sporangium. b, Groups of brown cells give a patchy appearance to *I. riparia* sporangium.

Pfeiffer (1922) and earlier workers refer to the presence or absence of bast bundles (fibers) in the peripheral tissues of the leaves. These tissues probably act as strengthening tissue and occur in plants as a result of specific ecological conditions. Pfeiffer found that all taxa in northeastern North America usually lacked bast bundles, except occasionally in emergent specimens of *I. eatonii* and *I. engelmannii*. All the other taxa are aquatic and the formation of bast bundles in the leaves never occurs. Because this feature is uncommon in the taxa under study, it has no taxonomic value. However this may be a useful feature in terrestrial and amphibious species. Of 62 taxa that Pfeiffer reviewed, all terrestrial species had peripheral bast bundles while those truly aquatic species mostly had none. Amphibious species, as expected, had few, weak bundles. Eaton (1900) and Clute (1905) also agree that the amount of bast depended on the exposure of the plant and that this character should not have very much importance in aquatic species.

Stomata are probably formed in developing leaves that are exposed to air. In most species studied here this does not occur regularly, because during early leaf development in spring, water is at a high level and plants are well covered with water, although they may become emergent later. This feature, like the bast

TABLE 4. Characters of the Sporangial Region in *Isoetes* spp.

	Sporangium			Velum	Ligule
	Length (mm)	Width (mm)	Color of mature sporangia	Length (mm)	Length (mm)
I. macrospora	3–5	3–4	clear to brown-spotted	1–1.5	to 2
I. hieroglyphica	5	3	usually clear	1	—
I. tuckermanii	3–5	3	clear to tan	1	to 2
I. riparia	3–7	2–4	clear to very pale tan groups or cells in streaks	to 2	to 3 (gen. less)
I. acadiensis	3–5	2.5–3	clear to single dark or pale tan cells scattered over sporangium	1–1.5	to 3
I. echinospora	4–10	3	clear, or patches of tan cells, or evenly tan	1–2.5	to 2.5
I. eatonii	6–12	3.4–5	clear or tan	1–2	to 3.5
I. engelmannii	3–10	2–4	clear	1–3	to 3.5

bundles, appears to be correlated with growing conditions. Species such as *I. macrospora* that grow in deeper water never have stomata. Those growing nearer to the shoreline occasionally show the presence of some stomata on leaves, as in *I. riparia* and *I. tuckermannii*, but in these it is not a constant feature (Eaton, 1900; Pfeiffer, 1922).

The length of membranous leaf margins that extend some distance up from the base of the leaf tends to be related to the length of the leaves and therefore indirectly to the vigor of the plants. This feature showed a great degree of variability from plant to plant within a given taxon. The maximum distance that this tissue reached on the leaves of most taxa was between 18 and 23 cm from the leaf base; however in most cases this distance was much shorter.

Characteristics of the Sporangial Region.—Sporangia vary in size and color depending on maturity or growing conditions. Young sporangia are unpigmented and appear transparent, while mature sporangia may or may not have some cells that have acquired a brown pigment. The number of cells affected by the pigment, their distribution, and intensity of the pigmentation determined whether the sporangium was scored as clear (no pigment), spotted (individual cells that are brown) (Fig. 3a), patchy (groups of brown cells in patches) (Fig. 3b), or tan (all cells slightly colored). Table 4 shows the sporangium coloration for all species, but data for *I. hieroglyphica* are based on relatively few specimens.

Matthews and Murdy (1969) found sporangial color patterns extremely variable in two southeastern species. This character tends to be constant for most specimens within species of the northeast, although occasionally there are striking exceptions to the otherwise typical patterns. *Isoetes eatonii*, *I. engelmannii*, *I. tuckermanii*, and *I. macrospora* had sporangia that were usually unspotted but a few specimens of *I. tuckermanii* had light brown or tan sporangia, and otherwise typical specimens of *I. macrospora* sometimes had sporangia that were

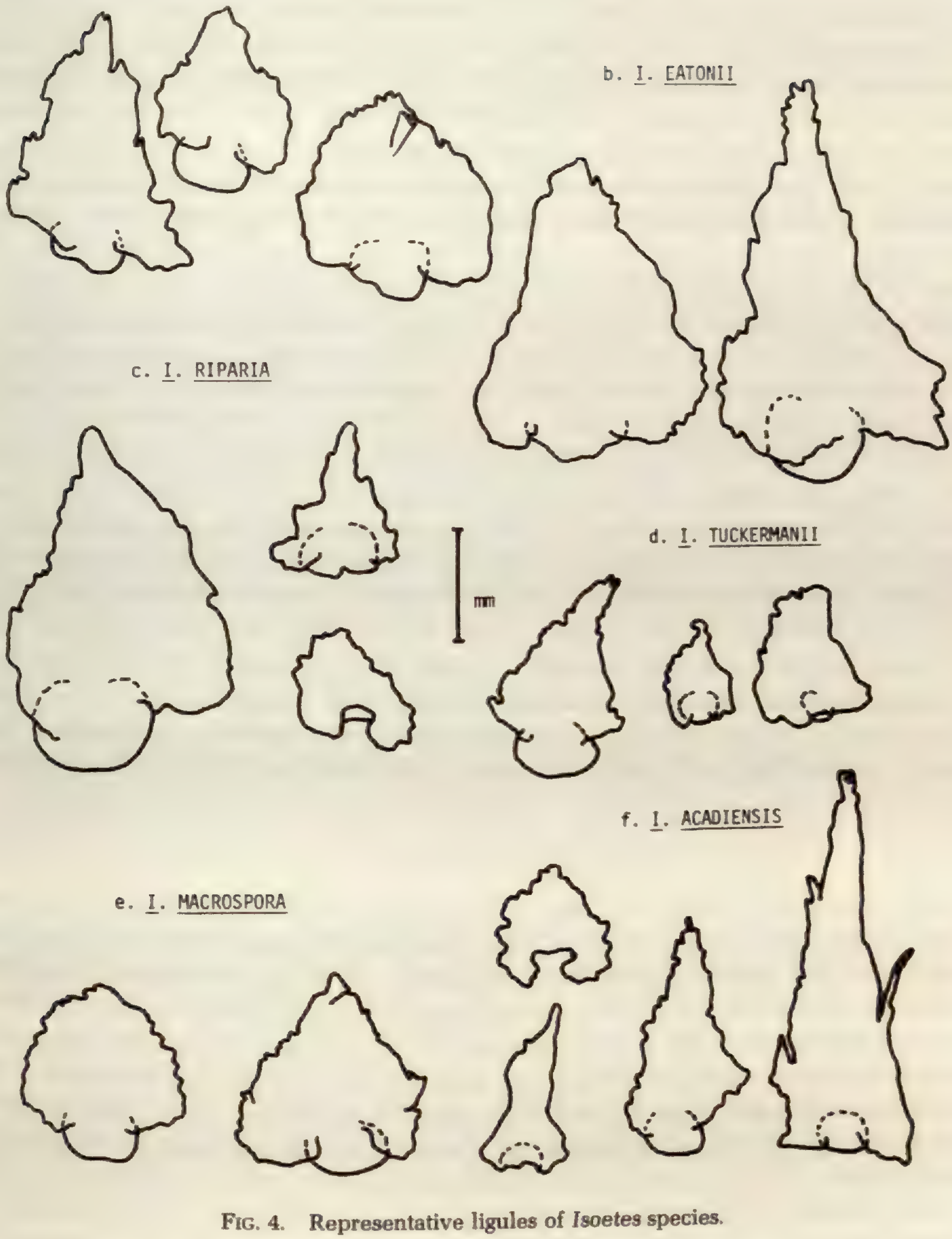


FIG. 4. Representative ligules of *Isoetes* species.

brown-spotted. *Isoetes echinospora* and *I. riparia* typically had colored cells in groups either sparsely or closely placed, which gave the sporangia a coarsely mottled aspect. *Isoetes acadiensis* usually had a tan or spotted sporangium wall.

Table 4 shows data collected on sporangium size for each taxon. Although *I. eatonii*, *I. engelmannii*, and *I. echinospora* appear to have the largest sporangia, the last generally had sporangia much smaller than 10 mm in length. Because *Isoetes eatonii* and *I. engelmanni* had large sporangia of generally the same size,

and the remaining taxa had smaller sporangia of comparable size, the character of sporangium size does not serve to differentiate individual taxa.

The velum is a thin layer of tissue that covers the upper regions of the sporangium on its adaxial side. This covering may be complete, almost complete, partial, or very short as in the species studied here. Velum lengths, measured from the base of the ligule downward towards the center of the sporangium, varied from less than 1 mm to 3 mm, but were generally 1.0–1.5 mm long. Therefore in these taxa this feature appears to be of no diagnostic value. Pfeiffer (1922) used a relative measure to determine velum length by relating it to sporangium length. Palmer (1896) found that vela varied from ¼ to ¾ of sporangium length on different leaves of one plant of *I. riparia*. Because the sporangia varied a great deal in size due to age of the leaf, while the vela generally retained a constant length within a given plant, this mode of measuring the velum length was considered unacceptable here.

Ligules—minute, fine flaps of tissue lying just above the sporangium on the leaf—were considerably damaged on dried specimens. Even ligules from living plants, which yielded the data for Table 4 and drawings for Figure 4, were so ephemeral that it was not certain whether the whole ligule was always observed. Although exact characterization of ligule shape and size is difficult, the ligules of *Isoetes echinospora* (Fig. 4a) and *I. macrospora* (Fig. 4e) were generally deltoid, being as long as wide; those of *I. riparia* (Fig. 4c) and *I. tuckermanii* (Fig. 4d) appeared to be triangular but slightly elongate, while ligules of *I. eatonii* (Fig. 4b) and *I. acadiensis* (Fig. 4f) usually appeared considerably elongate. Ligules of *I. eatonii* generally attained a larger size than the ligules of other species.

Conclusions

Most vegetative features of *Isoetes* are highly plastic and strongly reflect the growing conditions of the plants. This is also true for many other aquatics. Leaf length and leaf number have been statistically shown to have great variability within species and therefore are not diagnostic. Similarly the presence of stomata and bast bundles is a reflection of exposure to air during early leaf development. Perhaps leaf curvature and coloration may also be a product of environmental influences although this is difficult to prove. Nevertheless, these features show such variability within species that they cannot be of taxonomic or even diagnostic value.

Only sporangial coloration seems to generally correlate with other species characteristics (Kott & Britton, 1983). Although sporangium size appears to be somewhat constant within species, the size is often so similar among the taxa that it is not a valuable diagnostic aid. Both the velum and ligule do not yield further distinguishing information about these species.

It therefore must be concluded that vegetative features in *Isoetes* species of northeastern North America are either so constant (such as corm lobing and nature of roots) or so variable as to be of no taxonomic value. Species identification must rely primarily on spore characteristics (ornamentation and size), which agree well with other species specific characteristics such as chromosome

number, chromatographic spot patterns, ecological preferences, and behavior of the species.

Literature Cited

Clute, W. 1905. What constitutes a species in the genus *Isoetes*? Fern Bull. 13:41–47.

Eaton, A. 1900. The genus *Isoetes* in New England. Fernwort Papers 2:1–16.

———. 1908. Isoetaceae. Pp. 58–61 in *Gray's new manual of botany*, 7th ed. New York: American Book Co.

Fernald, M. L. 1950. *Gray's manual of botany*, 8th ed. New York: American Book Company.

Karrfalt, E. E. 1977. Substrate penetration by the corm of *Isoetes*. Amer. Fern J. 67:1–4.

Kott, L., and D. M. Britton. 1980. Chromosome numbers for *Isoetes* in northeastern North America. Canad. J. Bot. 58:980–984.

——— and ———. 1982a. Comparison of chromatographic spot patterns of some North American *Isoetes* species. Amer. Fern J. 72:15–18.

——— and ———. 1982b. A comparative study of spore germination of some *Isoetes* species of northeastern North America. Canad. J. Bot. 60:1679–1687.

——— and ———. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. Canad. J. Bot. 61:3140–3163.

Matthews, J. F., and W. H. Murdy. 1969. A study of *Isoetes* common to the granite outcrops of the southeastern Piedmont, United States. Bot. Gaz. (Crawfordsville) 130:53–61.

Palmer, T. C. 1896. Notes on *Isoetes riparia* and *Isoetes saccharata*. Bot. Gaz. (Crawfordsville) 21: 218–223.

Paolillo, D. J., Jr. 1963. The developmental anatomy of *Isoetes*. Illinois Biol. Monog. 31:1–130.

Peterson, R. L., M. Scott, and L. Kott. 1979. Root cap structure in *Isoetes macrospora* Dur. Ann. Bot. (London), n.s. 44:739–744.

Pfeiffer, N. 1922. Monograph on the Isoetaceae. Ann. Missouri Bot. Gard. 9:79–232.

American Fern Journal 75(2):56–70 (1985)

New Species of *Thelypteris* from Puerto Rico

GEORGE R. PROCTOR
Department of Natural Resources, P.O. Box 5887, Puerta de Tierra, PR 00906

During the course of field work in Puerto Rico since July 1983, many new fern records have been discovered, some of them representing entities new to science. Preliminary to publication of a book about Puerto Rican ferns, a series of short papers has been initiated in order to describe new taxa and discuss other relevant matters. A previous paper (Proctor, 1984) described a new species of *Trichomanes*; the present one concerns the genus *Thelypteris*.

All the newly discovered Puerto Rican thelypterids belong to subgenera *Amauropelta* and *Goniopteris*. In considering *Amauropelta*, the sectional assignments follow those of Smith (1974). There is no satisfactory modern classification of *Goniopteris*; therefore the species of this group are not assigned to sections in the present paper. In citing my own collections other than types, the first set of specimens is deposited in the herbarium of the Department of Natural Resources, San Juan, Puerto Rico (SJ); the second and third sets are deposited at the United States National Herbarium (US) and the Institute of Jamaica (IJ), respectively.

More than 100 species of *Thelypteris* occur in the Antilles, and nearly half of these belong to subgenus *Amauropelta*. The importance of these islands as centers of speciation for the latter taxon is emphasized by the recent discovery of three new Puerto Rican species, now being described. Two of these belong in section *Amauropelta*, and thus are allied to the species bearing sessile, reddish-resinous glands characterized many years ago by Morton (1963).

SUBG. *AMAUROPELTA* SECT. *AMAUROPELTA*

Thelypteris namaphila Proctor, sp. nov. (Fig. 1)—TYPE: Puerto Rico, Município de San Germán, Maricao State Forest, just S of Road 120 at approx. km 16.5, ca. 820 m, along banks of a small brook, 23 Nov 1983, *Proctor 39834* (holotype US; isotype IJ, SJ).

A *T. piedrensi* (C. Chr.) Morton squamis rhizomatis dense setulosis, planta multo minore et graciliore in omnibus partibus, lamina plene 2-pinnata cum numerosis pinnulis liberis nec adnatis nec decurrentibus, pinnulis auriculatis et inaequilateralibus et indusiis glandulis minutis albocapitatis exornatis differt.

Rhizome erect, slender (0.3–0.4 cm diam.), tightly invested with stipe bases; scales lustrous brown, narrowly deltate-acuminate, up to 4 mm long and 0.8–1.2 mm broad near base, minutely but abundantly setulose. Fronds erect, up to 45 cm long, of stiff texture; stipes 1.5–7 cm long, 0.5–1 mm diam., bearing near base a few spreading scales like those of rhizome, and minutely setulose on adaxial side and groove only, otherwise glabrous. Rhachis rather densely setulose on all sides throughout. Blades 2-pinnate, narrowly elliptic, up to 39 cm long, (4–) 7–

FLORA DE PUERTO RICO
Municipio de San Germán
Thelypteris namaphila Proctor, sp. nov.
MARICAO STATE FOREST: S. of Road 120, km. 16.5, elev. c. 820 m.
On moist banks of small stream in montane woodland; rhizome erect; fronds of stiff texture.
Fecha Nov. 25, 1963
0cm 1 2 3 4 5

FIG. 1. *Thelypteris namaphila*, holotype, *Proctor 39834*.

8.5 cm broad near middle, gradually narrowed toward base with 7–8 pairs of reduced, increasingly widely separated pinnae (the lowest mere auricles), acuminate at apex. Pinnae sessile, the longest linear-oblong, truncate or nearly so at the slightly inequilateral base, acuminate at apex, 0.5–1 cm broad, fully pin-

nate with up to 8 pairs of non-decurrent pinnules below the deeply pinnatifid apex; pinnules oblong-inequilateral, usually auriculate at base on acroscopic side, the margins otherwise entire to lightly crenulate, mostly up to 5 mm long and 1–1.5 mm wide, with 4–6 pairs of usually simple, very oblique veins, these strongly raised on both sides. Vascular parts and tissue minutely but harshly strigillose on adaxial side, the costae setulose beneath, the abaxial tissue glabrous and bearing scattered sessile reddish-resinous glands. Sori submarginal, confined to apical half of pinnules and segments; indusium small but persistent, ciliate with stiff acicular hairs, and also bearing a few minute whitish-capitate glands; sporangia glabrous.

It appears that *Thelypteris namaphila* was one of the elements originally included in *Aspidium sanctum* var. *portoricense* Kuhn (*Dryopteris sancta* var. *portoricensis* (Kuhn) C. Chr., Smithsonian Misc. Collect. 52:380. 1909). Of two isosyntypes at US one (*Sintenis 403*) is a mixture of *T. sancta* (L.) Ching and *T. namaphila*. The other isosyntype (*Sintenis 5956*), representing an isolectotype of var. *portoricense*, is entirely *T. sancta*.

In spite of its superficial similarity to *T. sancta*, *T. namaphila* is more nearly related to *T. piedrensis*, but differs from the latter in its more densely setulose rhizome scales, in being smaller and more slender in all its parts, in having fully 2-pinnate blades with many free pinnules neither adnate nor decurrent, with pinnules auriculate and inequilateral, and in its indusium with minute whitish-capitate glands (vs. indusium with much larger, sessile, globular, reddish-resinous glands). As its name implies, *T. namaphila* typically grows in wet soil beside brooks and streams, while *T. piedrensis* grows on stony slopes or shaded banks in montane forest. *Thelypteris namaphila* appears to be confined to an area of serpentine substrate, but *T. piedrensis* is not thus limited in distribution.

The delicate cutting of the blades of *T. namaphila* somewhat resembles that of *T. sancta* (for which it has been mistaken), but this new species clearly differs from *T. sancta* in its narrow, setulose rhizome-scales, its stiff, harsh texture, and in its submarginal sori with more prominent indusium.

Paratypes: PUERTO RICO. **Municipio de Maricao:** Monte Allegrillo, 900 m, 3 Apr 1913, *N. L. Britton et al. 2612* (US); Maricao State Forest, 700 m, 9 July 1963, *Liogier 9823* (US); Maricao State Forest, along Río Maricao above Criadero de Peces, 450–600 m, 18 Apr 1984, *Proctor 40468*.

Thelypteris rheophyta Proctor, sp. nov. (Fig. 2)—TYPE: Puerto Rico, Municipio de Ponce, Barrio Anón, along Río Inabón toward base of high falls, 500-700 m, among boulders beside stream, 21 Jan 1984, *Proctor 40042* (holotype US; isotypes IJ, SJ).

A *T. consanguinea* (C. Chr.) Proctor squamis rhizomatis fere deliquis, stipitis squamis late ovato-bullatis et cellulatis isodiametris tenuiter clathratis, pinnis paucioribus longius distantibus, pinnis maximis basi pinnulis duabus elongatis instructis, venis pluribus differt.

Rhizome erect or sometimes decumbent, 0.3–0.5 cm diam., closely invested with stipe bases, and nearly devoid of scales (those produced attached only to stipe bases). Fronds fasciculate, up to 45 cm long (often less than 25 cm); stipes

FLORA DE PUERTO RICO
Municipio de Ponce
Thelypteris rheophyta Proctor, sp. nov.
CORDILLERA CENTRAL: Barrio Anón. Along Río Inabón toward base of high falls, elev. 500–700 m.
Among boulders beside stream; rhizome erect.
Col. George R. Proctor No. 40042
Fecha Jan. 21, 1984
0cm 1 2 3 4 5

FIG. 2. *Thelypteris rheophyta*, holotype, *Proctor 40042*.

black at base, otherwise greenish-stramineous, 3–9.5 cm long, glabrous or nearly so, at first bearing toward base a few scattered, deciduous, broadly ovate-bullate, glabrous, delicately clathrate scales, the cells approximately isodiametric. Rhachis like the stipe, except for a line of minute incurved puberulence along adaxial

side. Blades oblanceolate, up to 33 cm long, 5–12 cm broad above the middle, gradually narrowed toward base (the lowest pinnae usually 0.5–1 cm long), long-acuminate at apex. Minute aerophores present at abaxial basiscopic base of pinnae. Pinnae usually 15–18 pairs, opposite, subopposite or alternate, inequilateral, usually ascending and somewhat falcate, with both proximal divisions (pinnules) longer than the rest, the basiscopic one usually at an angle divergent from the others on that side; longest pinnae typically 1.5 cm apart, narrrowly deltate-acuminate, mostly 2.5–6 cm long, 1–1.8 cm wide at base; segments approximately 7–12 on a side, oblong-falcate, acute at apex, mostly 1.2–1.5 mm wide, all but the free proximal pinnules narrowly joined at base with up to about 6 pairs of simple veins (to 7 in proximal pinnules, with basal veins in these usually forked). Costae hispidulous on adaxial side, the costules and tissue glabrous or with a few scattered hairs; all parts glabrous beneath, the tissue bearing few to rather numerous sessile, reddish-resinous glands. Sori supramedial to submarginal; indusium often asymmetric with one side slightly prolonged downward along vein, glabrous or sparsely hispidulous, the irregular margin usually beset with globular, reddish-resinous glands; sporangia glabrous.

Thelypteris rheophyta, as its name implies, shares with *T. consanguinea* and other related forms (including the recently described *T. fluminalis* A. R. Smith of Ecuador) the habit of growing along the rocky courses of streams, where the plants frequently become submerged in flowing water during periods of flooding. The group as a whole needs further taxonomic study. The Puerto Rican material now being described has usually been misidentified as *T. sancta*, but it is actually more nearly related to *T. consanguinea*.

I first thought these plants might represent a form of *T. consanguinea*, but further study has revealed an apparently consistent suite of characters distinguishing the Puerto Rican populations. *Thelypteris rheophyta* differs from *T. consanguinea* in the following characters: 1) near absence of scales on the rhizome, those at base of stipe being broadly ovate-bullate and delicately clathrate with nearly isodiametric cells, vs. obvious presence of an apical tuft of rhizome scales, these mostly deltate-lanceolate, flat, and with the clathrate cells consistently much larger, and longer than broad; in addition, the latter scales have resinous-glandular margins; 2) longer stipes (3–9.5 cm vs. 2–4 cm); 3) fewer pinnae (15–18 vs. 18–35) spaced farther apart (largest ones typically 1–1.5 cm apart, vs. 0.6–0.8 cm); 4) presence of a pair of free pinnules at base of largest pinnae (as well as on reduced pinnae toward base), the basiscopic one often longer than the acroscopic, and diverging at an angle from the other (and much shorter) divisions of the same side; in *T. consanguinea*, the proximal divisions of the longest pinnae are not free, and the basiscopic one is short and not divergent from the other segments on the same side (only on reduced pinnae toward base of blade are they longer and divergent); and 5) usually more numerous veins (4–7 vs. 3–5 pairs).

Thelypteris sancta is a plant of more delicate texture and cutting than *T. rheophyta*; its narrower, linear-oblong segments are usually more widely separated (often by more than their own width) by broad sinuses, and an indusial structure is absent or rudimentary. The scales at base of stipes are, however,

very similar. *Thelypteris rheophyta* occurs only along stream courses, but *T. sancta* is much more widely distributed.

From *T. resinifera* (Desv.) Proctor, which often grows in similar habitats, *T. rheophyta* differs in 1) the delicate, nearly isodiametric clathrate cell structure of the bullate-ovate scales near base of stipe (vs. elongate subclathrate cells of flat lanceolate scales, more like those of *T. consanguinea* but coarser); 2) fewer pinnae (15–18 pairs, vs. usually 30–40 pairs, seldom less); 3) presence of free proximal pinnules (vs. all divisions of the pinnae adnate); and 4) lamina glabrous (or nearly so) abaxially (vs. lamina pilosulous on vascular parts beneath).

Paratypes: PUERTO RICO. Without definite locality, May 1883, *Eggers 733* (US). **Municipio de Río Grande:** Sierra de Luquillo, Caribbean National Forest: Slopes of El Yunque, 28 May 1944, *W. H. Wagner s.n.* (US); Road 191, km 12.3, ca. 680 m, 8 Aug 1983, *Proctor 39373*; Road 191, km 9.9 at Quebrada Juan Diego, 480–500 m, 10 Oct 1983, *Proctor 39603*; Quebrada Sonadora above crossing of Road 186, 300–450 m, 21 Feb 1984, *Proctor 40172*. **Municipio de Arecibo:** Barrio Esperanza, vicinity of Observatorio de Arecibo, beside Río Tanamá, 160–180 m, 26 Feb 1984, *Proctor 40250, 40256*.

SUBG. *AMAUROPELTA* SECT. *UNCINELLA*

Thelypteris inabonensis Proctor, sp. nov. (Fig. 3)—TYPE: Puerto Rico, Municipio de Ponce, Cordillera Central, Toro Negro State Forest, along headwaters of Río Inabón above high falls, 1120–1250 m, 22 Jan 1984, *Proctor 40069* (holotype US; isotypes IJ, SJ).

A *T. rustica* (Fée) Proctor magnitudine multo minore, squamis setulosis, pilis plerumque acicularibus in rachi et abaxialiter in costis differt. A *T. frigida* (Christ) Smith & Lell. et *T. funckii* (Mett.) Alston squamis setulosis in stipite et rachi, pilis abaxialiter in costa crassioribus et numerosioribus et indusio dense longiciliato differt. A *T. frigida* stipitibus brevioribus, rachi pilis longis crassis pluricellularibus carenti, superficie adaxiali densius strigillosa differt. A *T. funckii* costa squamis carenti differt.

Rhizome erect, slender (ca. 0.5 cm diam.) and tightly invested with stipe bases, bearing toward apex numerous dark lustrous brown, narrowly lance-linear, densely setulose scales up to 4 mm long and 0.2–0.4 mm wide near base, the tips often contorted-filiform. Fronds erect-arching, up to 60 cm long; stipes 5–10 cm long, very densely hispidulous with grayish acicular (rarely hamate) hairs of variable length (mostly 0.2–0.4 mm long), and also beset with numerous spreading scales like those of rhizome; rhachis likewise clothed with similar hairs and scales throughout. Blades narrowly elliptic, up to 55 cm long, mostly 9–11 cm broad near middle, gradually narrowed toward base with 8–10 pairs of reduced, slightly deflexed pinnae (the lowest often less than 1 cm long), long-acuminate at apex. Pinnae mostly 25–30 pairs, sessile, the longest linear-oblong, truncate at base, acutish to short-acuminate at apex, 1.3–1.7 cm wide, pinnatifid with mostly 12–14 pairs of segments; aerophores absent. Segments oblong, slightly falcate, up to 6 mm long and 2–3 mm wide, rounded to subacute at apex, with up to 7 pairs of simple veins. Vascular parts and tissue densely strigillose on adaxial side; costae and costules densely hispidulous abaxially but lacking scales, the

FLORA DE PUERTO RICO
Municipio de Ponce
Thelypteris inabonensis Proctor, sp. nov.
CORDILLERA CENTRAL: Toro Negro State Forest.
Along headwaters of Rio Inabón above high falls,
elev. 1120-1250 m.
Moist eroding earth bank of stream in partial shade.
Jan. 22, 1984
0cm 1 2 3 4 5

FIG. 3. *Thelypteris inabonensis*, holotype, *Proctor 40069*.

tissue with numerous short, erect, acicular (rarely hamate) hairs and lacking glands. Sori small, supramedial to submarginal, often somewhat concealed by the narrowly reflexed margins, with persistent, densely long-ciliate indusium; sporangia glabrous.

This species seems related only to *T. rustica* in the West Indian area, but clearly differs from that Lesser Antillean species in its much smaller size, setulose scales, and in having mostly acicular (rather than hamate) hairs on rhachis and abaxially on costae. It more closely resembles *T. frigida* and *T. funckii* of Costa Rica and northwestern South America. From both of these it differs in its setulose scales, its stouter and more abundant abaxial costal hairs, and in its densely long-ciliate indusium. From *T. frigida* it further differs in its shorter stipes, in the intermediate and variable nature of the rhachis hairs (but none long, stout, and obviously pluricellular), and in being much more densely strigillose on the adaxial surface. From *T. funckii* it also differs in wholly lacking scales on the costae beneath.

Thelypteris inabonensis so far is known to occur only along the banks of a single stream, where it is uncommon. It should be considered a rare and endangered species.

SUBG. *GONIOPTERIS*

Thelypteris cordata (Fée) Proctor var. **imitata** (C. Chr.) Proctor, comb. et stat. nov.—*Dryopteris imitata* C. Chr., Kongl. Svenska Vetenskapsakad. Handl. III, 16(2):29, t. 6, figs. 1–4. 1937.—TYPE: Haiti, *Ekman H 3724*, isotype IJ. Range: Andros, Hispaniola, and Puerto Rico.

The small group of ferns allied to *T. cordata* presents a somewhat intricate problem in defining taxa. Christensen (1937) described *Dryopteris imitata* as "Near *D. cordata* (Fée) C. Chr. with similar pinnae but it is pinnate nearly to the apex, not proliferous, and with very conspicuous, reniform, grey indusia make it very distinct." However, subsequent accumulation of more material from a wider range tends to reduce the distinctiveness of these two forms. Populations in Cuba and Jamaica have numerous rather long, simple hairs on the rhachis (mixed with much shorter stellate ones) and a very minute indusium with simple and forked hairs; plants of the Bahamas (Andros), Hispaniola, and Puerto Rico bear only short stellate hairs on the rhachis and have a much larger indusium likewise covered with tiny stellate hairs only. Plants from all areas vary greatly in size (the Jamaican ones most consistently large), and there seem to be no other clear-cut morphological distinctions. Since neither typical *T. cordata* nor *T. imitata* are ever proliferous, Christensen's statement in this regard seems irrelevant except in relation to the allied *T. reptans* (J. F. Gmel.) Morton, which occurs throughout the same range. In my opinion, *T. cordata* and *T. imitata* should be considered only varietally distinct from each other.

Thelypteris cordata var. *imitata* is quite common on sheltered limestone cliffs in Puerto Rico, where its identity was first recognized by Weatherby on the basis of material collected by Chrysler in 1947 (US). Maxon (1947, p. 126) had previously misidentified other material (*Sargent 3273*, US) as *Dryopteris asplenioides* (Sw.) Kuntze.

Thelypteris abdita Proctor, sp. nov. (Fig. 4)—TYPE: Puerto Rico, Municipio de Utuado, Río Abajo State Forest, ca. 1.6 km due WSW of Campamento Crozier, 320–340 m, in sheltered or hidden crevices of moist vertical limestone cliff, 24 Jan 1984, *Proctor 40099* (holotype US; isotypes IJ, SJ).

0cm 1 2 3 4 5
FLORA DE PUERTO RICO
Municipio de Utuado
Thelypteris abdita Proctor, sp. nov.
RIO ARAJO STATE FORREST: c. 1.6 km. due W.S.W. of Campamento Crozier, elev. 320-340 m.
In sheltered crevices of moist vertical limestone cliff.
Jan. 24, 1984

FIG. 4. *Thelypteris abdita*, holotype, *Proctor 40099*.

A *T. cordata* var. *imitata* (C. Chr.) Proctor differt magnitudine multo minore, superficie stellato-puberula, venis simplicibus vel 1-furcatis, indusio glanduloso cum multis pilis simplicibus vel raro furcatis.

Rhizome erect, suberect, or decumbent, very small (0.2–0.4 cm diam.), short,

closely invested with stipe bases, at apex bearing a tuft of glabrous or sparingly stellate-puberulous lustrous brown scales, these narrowly deltate, acuminate, up to 4 mm long and 0.3–0.8 mm broad at base. Fronds few (usually 4–7), laxly fasciculate and spreading or decumbent, 6–19 (–23) cm long, forming a loose rosette; stipes stramineous, 2–7 cm long, 0.3–0.6 mm diam., lightly puberulous chiefly on adaxial side with minute stellate (sometimes a few merely forked) hairs. Rhachis more densely stellate-puberulous than stipe, on all sides. Blades approximately linear (rarely narrowly lance-linear), 5–16 (–19) cm long, 1–2 (–3) cm broad, pinnate nearly throughout, slightly narrowed at base end and at the blunt, shortly pinnatifid apex. Pinnae 10–20 pairs, mostly alternate, apart or sometimes approximate, stalked, rounded-oblong (rarely almost rotund) or very bluntly deltate-oblong, 0.5–1 (1.5) cm long, 0.3–0.6 cm wide, the margins entire, with 3–6 pairs of veins, these all simple or the basal 1–3 pairs forked, all tips free and ending in minute elliptic hydathodes short of the margin (seen adaxially). Vascular parts, margins, and tissue on both sides puberulous with few to numerous minute stellate hairs. Sori supramedial; indusium erect at sporangial maturity, irregularly roundish, with glandular margin and bearing numerous long, simple (rarely forked) hairs; sporangia glabrous.

This diminutive species has usually been mistaken in the past for a small form of *Thelypteris cordata* (Maxon, 1947, p. 127), and in fact grows with or near *T. cordata* var. *imitata* in many localities. However, many observations in the field have clearly shown that *T. abdita* is an independent, uniformly distinct, and much smaller entity, and bears little resemblance to *T. cordata* var. *cordata* as I have long known it in Jamaica. Comparison of *T. abdita* with *T. cordata* var. *imitata* shows that besides its much smaller size (mature fronds mostly 6–19 cm long and 1–2 cm wide, seldom larger, vs. up to 50 cm long and up to 5.5 cm or more wide), *T. abdita* differs in its densely to sparsely stellate-puberulous (vs. glabrous) tissue, simple or at most 1-forked veins, these always free (vs. many 1–2-forked veins, some often anastomosing), and moderate-sized, glandular indusium with many long, simple or rarely forked hairs (vs. a more prominent, non-glandular indusium with many much smaller, always stellate hairs). *T. abdita* is endemic to Puerto Rico; small forms of *T. cordata* var. *cordata* and *T. cordata* var. *imitata* seen from Cuba and Hispaniola respectively, though rather similar in appearance, are apparently juvenile developmental stages or else environmentally stunted, and are always distinct on the basis of one or more of the characters stated above. I am satisfied that *T. abdita* as described above represents the mature stage of this species.

Paratypes: PUERTO RICO. **Municipio de Utuado:** Utuado, 25 Mar 1887, *Sintenis 6588* (US); Utuado, 350 m, 12 Nov 1943, *Sargent 3272* (US). **Municipio de Lares:** Road 129 [old route], km 20, 7 May 1966, *Woodbury* s.n. (SJ). **Municipio de Quebradillas:** Barrio Charcas, near end of Road 437 (Finca Laboy), ca. 220 m, 15 Oct 1983, *Proctor 39625*. **Municipio de Arecibo:** Barrio Esperanza, gorge of Río Tanamá in vicinity of Observatorio de Arecibo, 200–270 m, 26 Feb 1984, *Proctor 40241*.

Thelypteris verecunda Proctor, sp. nov. (Fig. 5)—TYPE: Puerto Rico, Municipio de Quebradillas, Barrio Charcas, near end of Road 437 (Finca Laboy), ca. 220 m, on moist shaded limestone ledge near base of cliff, 5 Oct 1983, *Proctor 39581* (holotype US).

FLORA DE PUERTO RICO
Municipio Quebradillas
Thelypteris verecunda Proctor, sp. nov.
MONTAÑAS GUARIONEX: Barrio Charcas. Near end of Road 137, elev. c. 220 m.
On moist shaded limestone ledge near base of cliff.
Coll. George R. Proctor No. 39581 (holotype)
Fecha. Oct. 5, 1983
0cm 1 2 3 4 5

FIG. 5. *Thelypteris verecunda*, holotype, *Proctor 39581*.

A *T. reptanti* (J. F. Gmel.) Morton magnitudine minore, venis liberis plerumque simplicibus, indusii pilis longis simplicibus differt. *T. abditae* Proctor magnitudine et textura similis, sed ab illa frondibus dimorphis ubique pilis stellatis et simplicibus indutis, soris inframedialibus differt.

Rhizome creeping or decumbent, slender (ca. 0.2–0.3 cm diam.), bearing at

apex a small tuft of brown, minutely stellate-puberulous, deltate-acuminate scales ca. 1 mm long and 0.5 mm wide at base. Fronds decumbent-spreading, dimorphic, clothed throughout with minute stellate (occasionally forked) and numerous much longer simple hairs, the latter mostly 0.3–0.6 mm long; stipes 1–1.5 (–2) cm long, 0.4–0.5 mm diam. Sterile blades oblong, 2.5–4 cm long, 1.5–2 cm broad, truncate at base, rounded at the broadly lobed apex, with 2–4 pairs of short-stalked, round-oblong, entire pinnae, these 0.8–1 cm long and 0.4–0.6 cm wide; veins simple or 1-forked, all free. Fertile blades linear-attenuate, 13–15 cm (or more) long, 1.2–1.8 cm broad, truncate at base, the rhachis bearing a minute proliferous bud well below the apex; pinnae 15–20 pairs (or more), somewhat variable, mostly rounded-oblong to oval, 0.5–0.9 cm long. 0.3–0.4 cm wide, entire, short-stalked (or rarely a few sessile and somewhat adnate). Sori small, inframedial; indusium minute, erect, bearing a tuft of long, white simple hairs; sporangia glabrous.

The dimorphic fronds (the elongate ones proliferous), indument, and the inframedial position of the sori suggest comparison with *T. reptans*, but the small size, entirely free and mostly simple veins, and the long, simple indusial hairs indicate closer affinity with *T. abdita*. Perhaps it is a hybrid of these two species, both of which occur in the same general area. However, it is clearly distinct from both, and moreover appears to have normal, fertile sporangia.

Thelypteris verecunda so far is known only from the type collection.

Thelypteris hildae Proctor, sp. nov. (Fig. 6)—TYPE: Puerto Rico, Municipio de Utuado, Río Abajo State Forest, ca. 1.6 km due WSW of Campamento Crozier, 320–340 m, in crevices of moist shaded limestone cliffs, 24 Jan 1984, *Proctor 40100* (holotype US; isotypes IJ, SJ).

A *T. guadalupensi* (Wikstr.) Proctor habito cremnophilo, frondibus monomorphis stipitibus longioribus laminis basi truncatis, superficie glabra differt.

Plants always growing in crevices of shaded calcareous or non-calcareous cliffs or rock ledges. Rhizome short, erect or ascending, mostly 0.4–0.6 cm diam. (excluding the closely-investing stipe bases), at apex bearing a tuft of lustrous brown, glabrate or sparingly and minutely stellate-puberulous scales, these chiefly deltate-attenuate, up to 6 mm long and mostly 0.5–1 mm broad near base. Fronds few (usually 4–6), monomorphic, loosely fasciculate, spreading, up to 50 cm long (usually much less); stipes stramineous, (4–) 9–18 (–22) cm long (up to 45% of length of entire frond), 0.6–0.9 (–1.2) mm diam., minutely stellate-puberulous or sometimes glabrous. Blades narrowly lance-deltate or occasionally lanceolate, 12–28 (–32) cm long, 2.5–4.5 (–6.5) cm broad near base or below middle, truncate or very slightly narrowed at base, acuminate at apex, pinnatifid throughout (or sometimes a single pair of free pinnae, rarely more, at base) except toward the subentire apex. Segments 15–22 (–25) pairs, cut ½–⅔ (–¾) to the rhachis with mostly acute sinuses, oblong to narrowly deltate, blunt to acute at apex, with 8–17 pairs of veins, the majority of these (except in very large plants) simple and free (or a few 1-forked, these rarely anastomosing), the proximal pair from adjacent segments usually joined in the tissue and sending an excurrent veinlet

FIG. 6. *Thelypteris hildae*, holotype, *Proctor 40100*.

to the sinus. Adaxial side of blade glabrous except for minute stellate hairs along the rhachis-groove and sometimes a few on costae; abaxial side of blade with rhachis minutely stellate-puberulous to glabrous, and with scattered minute stellate hairs on costae and veins, the tissue glabrous; margins likewise with a few

stellate hairs especially at sinuses. Sori usually about medial, sometimes paired; moderately large indusium present, always minutely stellate-puberulous; sporangia glabrous.

The group of *T. guadalupensis* in Puerto Rico involves two common and distinct species usually lumped under one name. Typical *T. guadalupensis* (together with its somewhat enigmatic variant "forma *portoricensis*") is an essentially terrestrial species (sometimes also on mossy boulders, rarely on ledges), well known and widespread in the Greater and Lesser Antilles. It is common in Puerto Rico. Also in Puerto Rico occurs a related entity that is always found in the crevices of shaded cliffs and ledges, never in soil on the ground, and which is also morphologically distinctive. Although probably a close relative of *T. guadalupensis*, this entity, now described as *T. hildae*, differs consistently in 1) its habitat (always in crevices of ledges and cliffs, vs. nearly always terrestrial in soil or sometimes on mossy stones); 2) its monomorphic (vs. more or less dimorphic) fronds, these never proliferous at the tip; 3) its much longer, more slender stipes; 4) its blades truncate or but slightly reduced at base with seldom more than 1 pair of separated pinnae (if any). In *T. guadelupensis* the blades are long-decrescent downward, usually having two to many pairs of separate pinnae or widely-separated segments. In addition, *T. hildae* has glabrous (vs. minutely stellate-puberulous) tissue, and less divided, mostly free veins. This newly-recognized species is apparently endemic to Puerto Rico, where it has a wide distribution in suitable habitats. Unlike many species found on limestone rocks, *T. hildae* has also been found on cliffs of igneous origin.

Thelypteris hildae is named for Hilda Díaz Soltero, former Secretary of Natural Resources, under whose regime the present study of Puerto Rican ferns was initiated, and whose enthusiastic encouragement I have much appreciated.

Paratypes: PUERTO RICO. **Municipio de Utuado:** Probably near type locality, 7 Dec 1943, *Sargent 3298* (US); 22 Apr 1947, *Chrysler 6728* (US); 3 May 1983, *Acevedo 46* and *57* (SJ). **Municipio de Río Grande:** Sierra de Luquillo, Caribbean National Forest, Quebrada Juan Diego above Road 191, km 9.9, 480–500 m, 10 Oct 1983, *Proctor 39606*. **Municipio de Ponce:** Cordillera Central, Barrio Anón, along Río Inabón toward base of high falls, 500–700 m, 21 Jan 1984, *Proctor 40066*. **Municipio de Ciales:** Barrio Frontón, Road 140, km 49, ca. 320 m, 12 July 1984, *Proctor 40556*. **Municipio de Arecibo:** Barrio Esperanza, vicinity of Observatorio de Arecibo, 250–300 m, 26 Feb 1984, *Proctor 40238*. **Municipio de Hatillo:** Barrio Bayaney, Cueva Clara de Empalme, near intersection of Roads 134 and 455, 250–280 m, 25 Feb 1984, *Proctor 40188*; Cueva de la Catedral, near intersection of Roads 129 and 134, 200–250 m, 12 Nov 1983, *Proctor 39716*; same locality, 11 Aug 1984, *Proctor 40676*. **Municipio de Quebradillas:** Barrio Charcas, near end of Road 437 (Finca Laboy), ca. 220 m, 5 Oct 1983, *Proctor 39562*; gorge of Río Guajataca, E side of river along bottom of gorge, ca. 130 m, 15 Oct 1983, *Proctor 39610*.

Thelypteris hastata (Fée) Proctor var. **heterodoxa** Proctor, var. nov.—TYPE: Puerto Rico, Municipio de Hatillo, Barrio Bayaney, Cueva de la Catedral, near intersection of Roads 129 and 134, 220–250 m, on steep wooded slope in limestone ravine, 11 Aug 1984, *Proctor 40678* (holotype US; isotype SJ).

A *T. hastata* var. *hastata* lamina deltata vel ovato-deltata, pinnis latioribus marginibus lobatis differt.

Although identical in minute details, this entity differs from typical *T. hastata* in its deltate or ovate-deltate blades, the basal or next to basal pair of pinnae being the longest. This contrasts with var. *hastata*, whose blades are oblanceolate to narrowly oblong-obovate (rarely elliptic), being widest above the middle and normally having up to six gradually reduced pairs of pinnae toward base. Further, the pinnae of var. *heterodoxa* are usually 2.5 cm or more in width, with distinct marginal lobing, whereas those of var. *hastata* are seldom over 2 cm wide and are usually serrulate or crenate-serrate, or rarely shallowly lobed.

The group of *Thelypteris hastata* in Puerto Rico includes *T. hastata* itself (common), its var. *heterodoxa* (rare), *T. leptocladia* (Fée) Proctor, and several apparent hybrids involving these and other species. These forms require further investigation. However, the only other goniopterid fern in Puerto Rico with blades of somewhat similar outline to *T. hastata* var. *heterodoxa* is *T. abrupta* (Desv.) Proctor (recently discovered in this island for the first time, 26 Aug 1984, *Proctor 40762*), but the latter species clearly differs in details of indument, in the narrowly cuneate bases of the lower pinnae, in having a distinct, persistent indusium, and in the sporangial stalks (instead of the heads) being setulose. It is probably not closely related.

Acknowledgments

Several persons have provided greatly appreciated assistance thus far in my Puerto Rican fern work. Among these I would especially like to thank Dr. Juan "Tito" Cordero and his friends Miguel "Papo" Vives and William Estremera, of Quebradillas; and Dr. Horst Haneke and his delightful family, of Ponce. Among departmental colleagues, the field assistance of Barbara Cintrón, Oscar Díaz, Rubén Padrón, Benito Pinto, and Richard Studemundt has also been much appreciated. I thank Dr. Alain Liogier for assistance in preparing the Latin diagnoses, and Dr. José Vivaldi for reviewing the manuscript.

Literature Cited

Christensen, C. 1937. The collection of Pteridophyta made in Hispaniola by E. L. Ekman 1917 and 1924–1930. Kongl. Svenska Vetenskapsakad. Handl. III, 16(2):1–93.

Maxon, W. R. 1947. Puerto Rican fern notes. Proc. Biol. Soc. Wash. 60:123–130.

Morton, C. V. 1963. Some West Indian species of *Thelypteris*. Amer. Fern J. 53:57–70.

Proctor, G. R. 1984. A new filmy fern from Puerto Rico. Amer. Fern J. 74:7–8.

Smith, A. R. 1974. A revised classification of *Thelypteris* subgenus *Amauropelta*. Amer. Fern J. 64:83–95.

Shorter Notes

***Dryopteris ludoviciana* and *D.* ×*australis* New to Arkansas.**—Until recently, Arkansas was known to have three species and one hybrid of *Dryopteris* (Taylor & Demaree, Rhodora 81:503–548, 1979; Taylor, *Arkansas ferns and fern allies*, 1984), of which only *D. marginalis* (L.) Gray occurs frequently across the state. *D. spinulosa* (O. F. Muell.) Watt and *D.* ×*leedsii* Wherry are each known from one locality, while *D. celsa* (Palmer) Small is known from three localities. Consequently, the discovery in 1984 of a species and a hybrid new to the state constituted significant additions to the fern flora of Arkansas and furnished important phytogeographic data on the distribution of *Dryopteris* taxa in eastern North America (Carlson & Wagner, Contr. Univ. Mich. Herb. 15:141–162, 1982).

Dryopteris ludoviciana (Kunze) Small, Louisiana Log Fern, was discovered for the first time in Arkansas at Warren Prairie State Natural Area (Bradley Co.), located 20 km west of Monticello (*Peck & Peck 84641*, LRU, MICH, MIL; *Sundell & McIntyre 2864*, UAM). The fern grows with *Lorinseria areolata* (L.) Presl in the moist, lowland *Quercus phellos* L. woods. This Coastal Plain locality is 350 km north of St. Mary Parish, Louisiana, and Hardin Co. and Tyler Co., Texas, sites of the nearest previously reported populations (Thomas, Wagner & Messler, Castanea 38:269–274, 1979; Correll & Correll, *Aquatic and wetland plants of Southwestern United States*, 1972). The Arkansas population is the most northwestern in North America, and only the fourth population known to occur west of the Mississippi River.

Dryopteris ×*australis* (Wherry) Small, Southern Log Fern, the backcross hybrid between *D. celsa* and *D. ludoviciana*, was discovered for the first time in Arkansas at a wooded acid seep located at the southern periphery of the Ouachita Mts. in Garland Co., some 30 km southwest of Hot Springs (*Orzell 1429*, UARK; *Peck 84680*, LRU, MICH, MIL). The hybrid occurs with one parent, *D. celsa*, but not with *D. ludoviciana*. The closest known population of the latter species is at Warren Prairie, Bradley Co., Arkansas, 200 km to the southeast, making this hybrid population another example of "hybridization by remote control" (Wagner, Amer. Fern J. 33:71–73, 1943). The nearest hybrid populations occur in Louisiana, some 500 km to the southeast (Wagner & Musselman, Castanea 47:182–190, 1982). The Arkansas population is the most northwestern of the eight known populations of this North American hybrid. It is the third discovered and currently the only extant population west of the Mississippi River.

We thank W. C. Taylor, Milwaukee Public Museum, and W. H. Wagner, University of Michigan, for verification of our identifications. This research was sponsored, in part, by a faculty research grant from Office of Research and Sponsored Programs and the College of Science, Office of Research, Science and Technology, at University of Arkansas at Little Rock.—James H. Peck, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204; Steve L. Orzell, Arkansas Natural Heritage Commission, Little Rock, AR 72201; Eric Sundell, Department of Natural Sciences, University of Arkansas–Monticello, Monticello, AR 71655; and Carol J. Peck, Arkansas State Plant Board, Little Rock, AR 72203.

***Polystichum aleuticum* from Adak Island, Alaska, a Second Locality for the Species:**—Field work conducted by E. Hultén and W. J. Eyerdam in 1932 along the Aleutian Island chain resulted in many vascular plant collections reported in the *Flora of the Aleutian Islands* (Hultén 1937; ed. 2, 1960). *Polystichum aleuticum* C. Chr. in Hultén (Svensk Bot. Tidskr. 30:515, 1936) was collected by Eyerdam from Atka Island in the Central Aleutians. Until now, Eyerdam's collection has represented the only known occurrence of the species (Wagner, Pteridologia 1:1–64, 1979). It certainly must rank among the most restricted and rarest ferns of North America.

In 1975, while collecting bryophytes on Adak Island, I discovered a substantial population of this rare Aleutian fern. The specimens were examined and annotated by Wagner (ORE), who has indicated that they compare favorably with type materials. Specimens have been deposited in several herbaria: Alaska, Aleutian Islands, Adak Island, Mt. Reed, rock ledges below summit, 51°49′N, 176°44′W, 400 m, 19 Sept 1975, *D. K. Smith UT-54678* (ALA, ORE, TENN).

Christensen (in Hultén, Svensk Bot. Tidskr. 30:515–528, 1936; Amer. Fern J. 28:110–113, 1938) suggested a strong relationship exists among *P. aleuticum*, Himalayan *P. prescottianum* (Wall.) Moore, western and central Chinese species *P. shensiense* Christ, *P. sinense* Christ, *P. moupinense* (Franch.) Bedd, and *P. lachnense* (Hook.) Bedd. He noted, however, it is distinctive by "... its peculiar erose-dentate, greenish flat indusia and ... castaneous, thick stipes of older leaves ...." Christensen further noted that the only substantial difference between *P. lachnense* and *P. aleuticum* was the entire scales (vs. dentate scales) of the latter. Careful examination of the scales of Adak materials reveals that the scale margins are quite denticulate to shortly ciliate. Further studies are needed to clarify the relationship of the Aleutian fern to its Himalayan-Chinese counterparts.

Contributions from the Botanical Laboratory, University of Tennessee, n.s. No. 558.—DAVID K. SMITH, Department of Botany, University of Tennessee, Knoxville, TN 37996-1100.

BOOKS ON FERNS

An extensive list of floras, monographs, and horticultural manuals is available for two first-class stamps. I also buy used fern books; please send list of authors, titles, and dates. Myron Kimnach, 1600 Orlando Rd, San Marino, CA 91108.

BRITISH PTERIDOLOGICAL SOCIETY

Open to all who are interested in growing and studying ferns and fern-allies. Full members receive THE FERN GAZETTE and the BULLETIN OF THE BRITISH PTERIDOLOGICAL SOCIETY. Membership subscriptions are £ 7 for full members, £ 5 for ordinary members (not receiving the GAZETTE), and £ 5 for student members (under 25 years of age). For particulars, U.S. residents should apply to Dr. J. Skog, Biology Department, George Mason University, Fairfax, VA 22030. Non-U.S. residents should apply to Hon. Gen. Sec., % British Pteridological Society, British Museum (Natural History), Cromwell Road, London SW7 5BD, England.

AMERICAN FERN JOURNAL

Volume 75

Number 3

July–September 1985

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

An Unusual Submerged Aquatic Ecotype of *Asplenium unilaterale*
Masahiro Kato and *Kunio Iwatsuki* 73

A Newly Discovered Habitat for *Isoëtes melanopoda* in Louisiana *R. Dale Thomas* 77

Anatomical Studies of *Sphaeropteris* and *Cnemidaria* (Cyatheaceae)
Terry W. Lucansky 00

Germination and Morphology of Spores of *Trichipteris corcovadensis*
Luciano M. Esteves, Gil M. Felippe, and *Therezinha S. Melhem* 92

Shorter Notes

A New Station for *Dicranopteris flexuosa* in Bay County, Florida
James R. Burkhalter 79

New Locations for *Isoëtes tegetiformans* in Georgia *Phillip M. Rury* 102

Reviews 76, 104

Information for Authors Cover 3

MISSOURI BOTANICAL

DEC 17 1985

GARDEN LIBRARY

The American Fern Society

Council for 1985

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268. *President*

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH . . . . . Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY . . . . . Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER . . . . . Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER . . . . . U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER . . . . Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $9.00 gross, $8.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $8.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $160.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 75(3):73-76 (1985)

An Unusual Submerged Aquatic Ecotype of *Asplenium unilaterale*

MASAHIRO KATO
Botanical Gardens, Nikko, Faculty of Science, University of Tokyo, Nikko 321-14, Japan
KUNIO IWATSUKI
Botanical Gardens, Faculty of Science, University of Tokyo, Tokyo 112, Japan

Although many ferns inhabit moist places, extremely few are actually deserving of the term aquatic. There are the floating miniferns *Azolla* and *Salvinia*, and others that usually root in mud with a fluctuating water level, e.g., *Ceratopteris*, *Acrostichum*, and Marsileaceae. Rheophytes, subject to floods in streambeds, are periodically aquatic. Some plants are moistened with constant spray from waterfalls (Giesenhagen, 1892; Mickel, 1972; Iwatsuki, 1975; Page, 1979). A species of *Stenochlaena* lives in water pockets formed by *Pandanus* leaf bases (Price, 1982, p. 200).

This report of *Asplenium unilaterale* Lam. makes it the second known fern that lives as a genuine submerged aquatic. The other such is *Bolbitis heudelotii* (Fée) Alston of Africa, sometimes cultivated in tropical aquaria, although it is merely a form of the species that is most often rheophytic (Hennipman, 1977, p. 236–240). *Asplenium unilaterale* is already known to be highly variable in cytology (Lovis, 1973), reproduction (Murakami & Iwatsuki, 1983) and habitat and morphology (Iwatsuki, 1975), with some plants (var. *udum*) having an extremely thin (bistratose) lamina lacking intercellular spaces.

The submerged form was collected in Seram (Ceram) Island, the Moluccas, Indonesia, during field trips in 1983 and 1984–85. On the island, ecologically and reproductively different forms of *Asplenium unilaterale* also are terrestrial or on dry or wet rocks in montane forests, as they are in the entire range. The new form grows in a spring named "Air Mata Makariki" at 350 m above sea level on the southern slope of Murkele Mountain Ridge at the central part of the island. This area is abundant in limestone rocks, and river water occasionally underflows. The spring is formed at the mouth of an underflowing stream and water flows out with air bubbles from the bottom of the spring at several points and goes down rapidly along Wae (=River) Makariki (Fig. 1). The water level appeared to be constant and the water temperature measured 19°C. The spring is surrounded by steep slopes covered by semi-primary forest. *Asplenium subnormale*, a lowland species closely related to *A. unilaterale*, was found growing terrestrially on the slope, but *A. unilaterale* was absent there.

The submerged form consists of several populations, and all the individuals are uniform in morphology. The largest population grows submerged 50–70 cm (to the top of the leaves) below the water surface in rather slowly flowing water, with numerous air bubbles, and epiphytic on likewise submerged roots of two trees of *Ficus* sp. (one ca. 28 cm and another 33 cm in DBH) which stand in the spring (Fig. 1). Smaller populations grow more shallowly submerged on roots

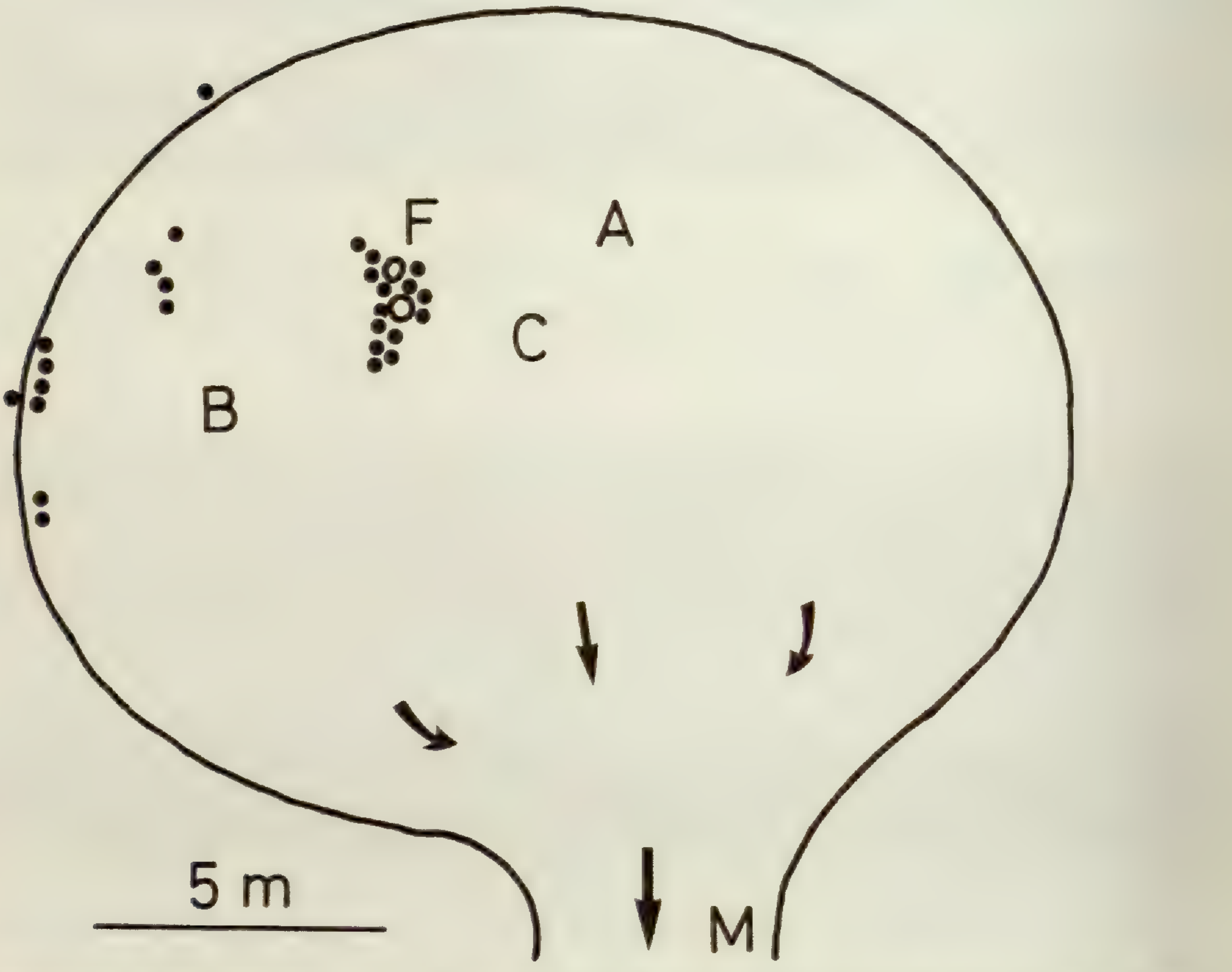
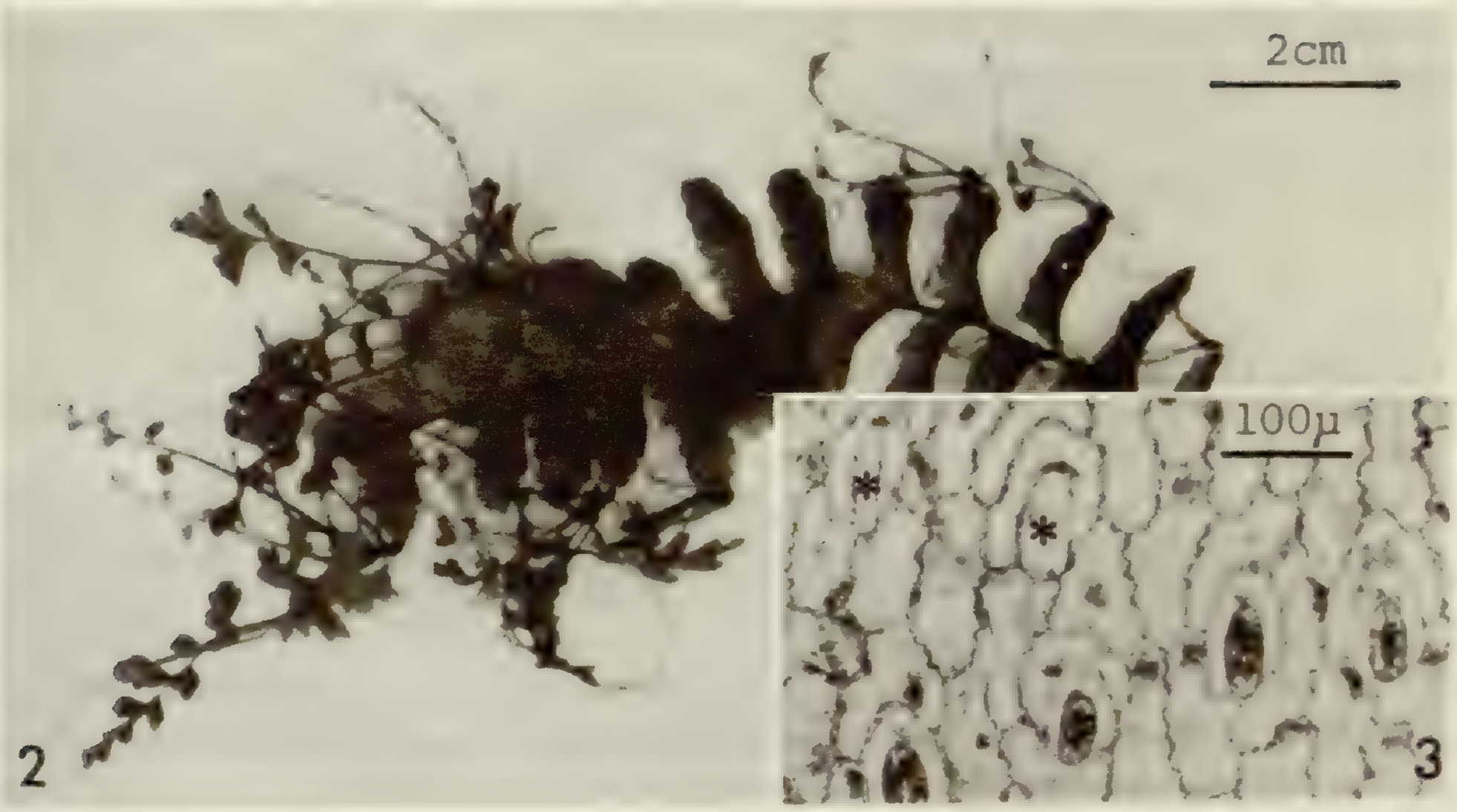


FIG. 1. Diagram of the spring "Air Mata Makariki." A, B: two largest water-flowing mouths. C: the deepest point (ca. 2 m deep). F: two trees of *Ficus* sp. M: Wae (=River) Makariki. Solid circle: individuals of the submerged aquatic ecotype of *Asplenium unilaterale*. Arrows indicate water flow.

from trees on the slope, or among rocks at the bottom of the spring. The smallest populations, which consist of a few individuals, grow on tree roots and in rock crevices, both beside the spring, slightly above the water surface.

The submerged form differs from the terrestrial only in the following combination of characters: rhizomes are rather long-creeping, branching, and bearing long slender branched roots, altogether constituting an entangled rhizome-root system. Detached leaves or pinnae are trapped by the rhizome-root system, and new plants anchor and grow in the system (Fig. 2). Anatomical features of the roots do not differ from those of the terrestrial forms. Gemmae are profusely borne and develop into young plants on pinnae, whereas the terrestrial forms are usually not gemmiferous. However, plants that were transplanted in pots in a greenhouse in the University of Tokyo Botanical Gardens in 1983 have so far not produced any gemmiferous leaves.

In the aquatic form, the apices of pinnae are broadly obtuse as compared with subacute apices of the land forms. Anatomically the pinnae consist of an upper epidermis, a mesophyll of spongy tissue and a lower epidermis with stomata, as in the land forms, but 10–20% of stomata are incomplete (Fig. 3). Newly developed leaves of two-year-old plants cultivated in soil in pots retain this feature. Each sporangium, a typical leptosporangium of the terrestrial *Asplenium unilaterale*, produces approximately 32 spores, some of which are abortive, with the



FIGS. 2, 3. Submerged aquatic *Asplenium unilaterale*. FIG. 2. Leaf bearing young plants developed from gemmae. FIG. 3. Surface view of a lower epidermis. Asterisks indicate incomplete stomata.

same exospore ornamentation as that of the terrestrial form. The number suggests an ability to be apogamous.

In contrast to species of aquatic ferns that are distinct morphologically and taxonomically, the submerged *Asplenium unilaterale* retains the morphology for land life and is considered to be an ecotype or a local form at the early stage of quantum speciation (Grant, 1981). The peculiarly submerged aquatic life seems to be facultative in the presumably recently formed habitat. This assumption is based on the small morphological differences such as incomplete development of some stomata between the submerged and land forms, and the recent origin of the spring which is suggested by the occurrence of the *Ficus* sp. trees in the spring.

Another noteworthy aspect is reproduction of the submerged form. The vegetative reproduction by pinna-borne gemmae that grow into new plants anchoring in the rhizome-root system may be the most suitable means for the fern in question to remain completely submerged in the flowing water. It is a substitute for reproduction by spores and gametophytes which, even if apogamous, might not be possible for this fern, because freely dispersed spores are easily washed away in flowing water. Since such vegetative reproduction occasionally takes place in the terrestrial *Asplenium unilaterale* in Seram Is., it is a phenotypic variable that is merely strongly expressed by the aquatic form. A parallel development is the possible induction of adventitious buds in a saturated atmosphere in the filmy fern *Trichomanes minutum* (Yoroi & Iwatsuki, 1977).

The origin of the submerged form remains to be clarified, but it may be suggested that when the spring arose, the aquatic ecotype was derived from one of the possibly apogamous land forms that occur on Seram Island. Biosystematic analysis of interrelationships among the infraspecific variants of *Asplenium unilaterale* on the island is needed.

This study was financially supported by Grant-in-Aid for Overseas Scientific

Field Research from the Ministry of Education, Science and Culture, Japan, nos. 58042006, 59041021 and 60043021.

Literature Cited

Giesenhagen, K. 1892. Ueber hygrophile Farne. Flora 76:157–181.

Grant, V. 1981. *Plant speciation*. 2nd ed. New York: Columbia University Press.

Hennipman, E. 1977. A monograph of the fern genus *Bolbitis* (Lomariopsidaceae). Leiden Bot. Series 2:1–331. The Hague: Leiden University Press.

Iwatsuki, K. 1975. Taxonomic studies of Pteriodophyta X. Acta Phytotax. Geobot. 27:39–55.

Lovis, J. D. 1973. A biosystematic approach to phylogenetic problems and its application to the Aspleniaceae. Pp. 211–228 in *The phylogeny and classification of the ferns*, ed. A. C. Jermy et al., J. Linn. Soc. Bot. 67 (Suppl. 1).

Mickel, J. T. 1972. A "filmy fern" in the genus *Cystopteris*. Amer. Fern J. 62:93–95.

Murakami, N. and K. Iwatsuki. 1983. Observation on the variation of *Asplenium unilaterale* in Japan with special reference to apogamy. J. Jap. Bot. 58:257–262.

Page, C. N. 1979. The diversity of ferns. An ecological perspective. Pp. 9–56 in *The experimental biology of ferns*, ed. A. F. Dyer. London: Academic Press.

Price, M. G. 1982. The ferns of Steer and Harrington. Contr. Univ. Michigan Herb. 15:197–204.

Yoroi, R. and K. Iwatsuki. 1977. An observation of *Trichomanes minutum* and allied species. Acta Phytotax. Geobot. 28:152–159.

Review

"*Index filicum. Supplementum quintum: pro annis 1961–1975*," by F. M. Jarrett, with collaborators. 1985. 245 pp. New York: Clarendon Press, Oxford University Press. ISBN 0-19-854579-7. $39.95.

Students of ferns are familiar with the great value of this index, begun by that most illustrious of pteridologists, Carl Christensen, in 1905. It is the only comprehensive source for obtaining bibliographic citations (name and place of publication, plus basionym for new combinations and locality for new species) for all Latin names, generic rank and above. Thus, we are forever grateful to the compilers of this and all preceding volumes. A new and welcome feature of the fifth supplement is the inclusion of names of fern allies. Corrections from past indices are denoted by an asterisk. As helpful as this index will be, it will not satisfy all needs: continuing the tradition of past volumes, infraspecific names are not indexed. In evaluating genera with which I am most familiar, I found only a few minor errors and omissions (to whom should I send them?) that will no doubt be corrected in subsequent volumes. One parting lament—one wishes that this index were more current: in the last ten years, I extrapolate that enough new names have accumulated to fill about 165 pages of a new supplement!—Alan R. Smith, Department of Botany, University of California, Berkeley, CA 94720.

American Fern Journal 75(3):77–79 (1985)

A Newly Discovered Habitat for *Isoëtes melanopoda* in Louisiana

R. Dale Thomas

Herbarium, Department of Biology, Northeast Louisiana University,
Monroe, Louisiana 71209-0502

Isoëtes melanopoda Gay & Dureau, Blackfoot Quillwort, ranges from Georgia to Texas and north to South Dakota and New Jersey. Old collections are known from East Baton Rouge, Avoyelles, and Rapides parishes in Louisiana (Brown & Correll, 1942). More recent collections have been made from Calcasieu (Brooks & Maples, 1971) and Sabine (Carroll & Thomas, 1981) parishes. However, this species has been considered rare in the state (Thieret, 1980) and until the present finds the only extant population known in Louisiana was from a low woods in Sabine Parish. Recently, plants of *I. melanopoda* were found to be locally abundant and quite common in wet, low areas of unplowed soybean and cotton fields.

The new habitat for *I. melanopoda* in Louisiana was discovered on 8 May 1984. A low wet area in an unplowed soybean field in Richland Parish provided the first find. All the surrounding parts of the field had been treated with herbicides to kill the winter weeds in preparation for spring planting. The low area was too wet for tractors to negotiate so it had been left unsprayed. Close examination of the area revealed that literally tens of thousands of plants of *Isoëtes melanopoda* were thriving in the field. Many plants in the treated areas showed no ill effects of the herbicide, although all seed plants were dead. A week later the entire field, including the low area, was disced and planted in soybeans.

During the period of a month I found new populations in Richland, Ouachita, Morehouse, Caldwell, Catahoula, West Carroll, Bossier, and Caddo parishes. All these populations contained plants that were much larger, more numerous, and less scattered than those previously collected from Sabine Parish (Carroll & Thomas, 1981). All populations found in the nine new parishes were either in cultivated fields (soybean—West Carroll, Richland, Catahoula; cotton—Morehouse; oat—Franklin; or open wet areas like pastures or hayfields—Caldwell, Ouachita, Bossier, Caddo). The areas might be flooded during heavy winter rains but in no areas were plants found completely submerged in water. The areas are dominated by *Juncus* and Cyperaceae with a few dicotyledons. The most abundant *Juncus* species are *Juncus biflorus*, *J. bufonius*, *J. diffusissimus*, *J. dudleyi*, *J. scirpoides*, *J. validus*, and rarely *J. effusus* or *J. coriaceus*. Cyperaceae is represented by *Carex frankii*, *Cyperus iria*, *C. pseudovegetus*, *Fimbristylis autumnalis*, *F. miliacea*, *F. vahlii*, *Scirpus koilolepis*, and undetermined species of *Eleocharis*. Dicotyledons included *Tillaea aquatica*, *Plantago hybrida*, *P. virginica*, *Callitriche peploides*, *Cerastium glomeratum*, *Ranunculus pusillus*, *Gratiola neglecta*, *G. virginca*, *Lindernia anagallidea*, and *Linaria canadensis*. The sterile low specimens of *Eleocharis* and *Juncus* make the *Isoëtes* specimens very difficult to locate at first. Concentrated searching reveals that *Isoëtes melanopoda*

leaves are more pea-green than those of the seed plants. Leaves of *Isoëtes* are splayed in the open; this character is lost in thick vegetation.

Current cultivation practices for cotton and soybeans seem to favor the spread of *Isoëtes melanopoda* in the South. When a farmer begins to prepare his fields for planting in the spring, low, wet areas are left to be disced later after further drying. By the time these low areas are dry, spores of *Isoëtes* are mature. The spores spend the summer in the ground and apparently germinate only after fall rains begin. The corms of old plants are also dormant during the summer and new leaves appear again in November. Denser populations of *I. melanopoda* occur in cultivated areas than in uncultivated ones. In the Franklin Parish population, plants were in distinct rows following the furrows left by individual discs. All populations in Ouachita, West Carroll, and Franklin parishes checked in December, 1984 had thousands of plants from old corms bearing leaves about four inches tall. These leaves grew to about 10 inches in length by April 1985 and were withered and dead by 1 June 1985. Almost all cotton and soybean fields in this area lay fallow from harvest time in October and November until the following April or early May. Although *Isoëtes* is a perennial plant, its summer dormancy allows it advantages similar to those of winter annuals. Recent improvements in laser technology have made it possible for farmers to have their fields leveled and sloped to prevent low areas. No populations of *Isoëtes* were found in these fields, perhaps because they can now be disced earlier and thus before the spores have had a chance to mature.

Collections made during 1984: LOUISIANA.—**Richland Parish:** Unplowed soybean field S of La. 15 on Richland Plantation, 1.5 miles W of Rhymes and La. 133, Sec. 10, T16N, R5E, 8 May, *Thomas 88366*. **Franklin Parish:** Edge of oat field W of dirt road, S of La. 132 and Old Mixon School near Murphy woods, Sec. 15, T16N, R8E, 8 May, *Thomas 88417*. **Sabine Parish:** Wet woods beside U.S. 171 at Bayou San Patricio, about 2.8 miles NW of Noble and La. 483, Sec. 33, T9N, R13W, 15 May, *Thomas 88666 & Taylor 6956*. **Ouachita Parish:** Low wet area of pasture on Layton Farm, S of U.S. 80 at Rifle Range Road E of La. 139 and Sicard, E of Monroe, Sec. 71, T18N, R4E, 23 May, *Thomas 88835*. **Morehouse Parish:** Wet, low unplowed area in soybean field beside paved road N of Little Lake Lafourche and La. 595 and S of Gum Ridge, Sec. 21, T18N, R6E, 23 May, *Thomas 88846*. **Caldwell Parish:** shallow, temporary pool at gas pipe line beside La. 133, 4.4 miles N of La. 847 N of Hebert, Sec. 31, T15N, R5E, 24 May, *Thomas & Earthman 88879*. **Catahoula Parish:** Wet area at edge of field at ball park beside La. 124, S edge of Harrisonburg, Sec. 38, T9N, R6E, 24 May, *Thomas & Earthman 8886*. **West Carroll Parish:** Low end of soybean field S of La. 2 at Redwing near Big Colewa Bayou W of Oak Grove, Sec. 13, T21N, R9E, 25 May, *Thomas 88904*. **Bossier Parish:** Low edge of hay field in creek bottom N of La. 2 at branch of Cypress Bayou, 1.1 miles E of La. 157 and E of Plain Dealing, Sec. 12, T22N, R13W, 1 June, *Thomas 88994 & Taylor 7166*. **Caddo Parish:** Low, open wet area dominated by *Juncus* near a small stream on Kendrick Road W of La. 1 and 2.7 miles N of Vivian, Sec. 11, T22N, R16W, 1 June, *Thomas 89029 & Taylor 7201*.

Voucher specimens of all collections are on deposit in Northeast Louisiana University Herbarium (NLU). Duplicates of some of the collections are available for exchange.

LITERATURE CITED

BROOKS, J. H. and R. S. MAPLES, JR. 1971. A recent find of *Isoetes* in Louisiana. Amer. Fern J. 61: 186.

BROWN, C. A. and D. . S. CORRELL. 1942. *Ferns and fern allies of Louisiana*. Baton Rouge: Louisiana State University Press.

CARROLL, A. N. and R. D. THOMAS. 1981. *Isoetes melanopoda* in Sabine Parish, Louisiana. Phytologia 48:274–275.

THIERET, J. W. 1980. *Louisiana ferns and fern allies*. Lafayette: Louisiana Natural History Museum.

SHORTER NOTE

A New Station for *Dicranopteris flexuosa* in Bay County, Florida.—While performing a routine botanical field survey at Bay Point Resort near Panama City, Florida, on 7 November 1984, I discovered a small colony of *Dicranopteris flexuosa* (Schrad.) Underw. (forked fern or net fern) in a shallow empty drainage ditch passing through a slash pine forest southeast of the junction of Delwood Beach Road and Magnolia Beach Road. The plants were situated at the upper edge of the ditch slope, were rooted in soil consisting of whitish sand and dark organic particles, and grew with *Lycopodium cernuum* and *Woodwardia areolata*. This habitat is similar to other sites where *D. flexuosa* has previously been found in the southeastern United States (Wherry, *The Southern fern guide*. 1964; Lakela & Long, *Ferns of Florida*. 1976).

This gleicheniaceous fern, which is native to Mexico, South America, and the West Indies, was first collected in the United States in 1913 in Mobile County, Alabama, but did not persist there (Small, *Ferns of the Southeastern States*. 1938; Dean, *Ferns of Alabama*, rev. ed. 1969). Likewise, in 1947 it was discovered in a transient colony in Osceola County, Florida, and in 1955 a colony was found in Hillsborough County, Florida, that was still extant in 1964 (Wherry, op. cit.). Lakela and Long (op. cit.), however, reported no recent collections of this fern. The present account is thus the first documented report of *D. flexuosa* in the United States in 20 years, and the Bay County station constitutes an extension of 400 kilometers northwestward from the last previously documented site in Hillsborough County. *Dicranopteris flexuosa* is also hereby verified as an extant natural element of the pteridophyte flora of Florida and the United States. Owing to its obvious rarity, its inclusion on Florida's rare and endangered biota lists is certainly warranted. Herbarium specimens (*Burkhalter 9784*) have been deposited at UWFP, FSU, and FLAS.

Dr. Michael I. Cousens, a pteridologist from the University of West Florida, visited the Bay County *Dicranopteris* colony on 19 January 1985 and collected frond material for gel electrophoretic analyses. He also discovered a number of *Dicranopteris* gametophytes at that time, which possibly indicates that this small isolated colony of this interesting tropical fern is reproducing in the seemingly nonsalubrious climate of northwest Florida.—JAMES R. BURKHALTER, The Herbarium, Building 58, Room 77, University of West Florida, Pensacola, FL 32514.

American Fern Journal 75(3):80–91 (1985)

Anatomical Studies of *Sphaeropteris* and *Cnemidaria* (Cyatheaceae)[1]

TERRY W. LUCANSKY
Department of Botany, University of Florida, Gainesville, FL 32611

Despite a renewal of interest in the tree ferns (Tryon, 1970, Gastony, 1973, 1974, 1981; Gastony & Tryon, 1976; Stolze, 1974), relatively little is known anatomically about these largest of ferns. Previous anatomical studies have dealt primarily with mature paleotropical species (Bower, 1912; Ogura, 1927, 1972; Godwin, 1932; Mehra & Singh, 1955) and have shown that similarities between genera occur in both the vascular anatomy and nodal patterns. Recent studies of mature members of the neotropical Cyatheaceae also have shown striking similarities among the genera (Lucansky, 1974b, 1976b, 1977; Lucansky & White, 1974).

Tryon (1970) revised the classification of the family Cyatheaceae and recognized six genera and three principal evolutionary lines among the squamate genera. *Sphaeropteris* with its undifferentiated (conform) scales represents an evolutionary line and occurs at the base of the squamate genera, while *Cnemidaria* with its marginate scales is found at the top of another evolutionary line. *Sphaeropteris* supposably contains elements allied to the other two major evolutionary groups in the family (Tryon & Tryon, 1982). On the basis of scale characters, *Sphaeropteris* subgenus *Sphaeropteris* is related to *Alsophila* and subgenus *Sclephropteris* is allied with *Trichipteris*, *Cyathea* and *Cnemidaria*. Holttum and Edwards (1983) believe that this latter alliance (together with certain species of Tryon's *Sphaeropteris*) forms a natural group which needs a new subdivision.

Holttum (1963, 1964, 1965) also has intensively studied the Cyatheaceae, but recognized only a single genus *Cyathea* for the same taxa in the family. He subdivided the genus on the basis of characters of the stipe scales and associated characters of indusia, hairs and venation, and felt that the only sharp subdivision within the genus is between the subgenus *Sphaeropteris* and the subgenus *Cyathea* (Holttum, 1982; Holttum & Edwards, 1983). His subgenus *Sphaeropteris* is characterized by peculiar marginal setae on the stipe scales and certain indusial and venation characters, while Tryon (1970) regarded the uniformity of cells of the stipe scales as the most important character for delimiting the genus *Sphaeropteris*. Although Tryon (1970) promoted subgenus *Sphaeropteris* to generic rank, Holttum & Edwards (1983) feel that it is not a natural group and contains species that do not belong in the group.

Sphaeropteris sensu Tryon is a genus of approximately 120 species found in both the New and Old World. Its distinctive feature is the presence of conform stipe scales with undifferentiated or poorly differentiated cellular construction

---

[1] Publication No. 6244 of the Florida Agricultural Experiment Station.

(Tryon, 1970). In some respects (e.g. leaf morphology, position of trichomes and indusium) this genus is similar to the other squamate genera in the Cyatheaceae.

Cnemidaria sensu Tryon, is a genus of 25 species confined to the American tropics. Its closest relationship is with *Cyathea* (Tryon & Tryon, 1982), and Holttum even includes it in subgenus *Cyathea* of the genus *Cyathea* (Holttum & Edwards, 1983). According to Tryon (1970) this genus constitutes a strong evolutionary line, and several characters, including the lack (rarely present) of trichomes on the adaxial side of the costae, contrast markedly with the other squamate genera. Based upon spore morphology, venation, leaf architecture, habit and lack of trichomes adaxially on the costae, *Cnemidaria* forms the most natural and distinctive genus in the Cyatheaceae (Stolze, 1974). Holttum & Sen (1961) reinforced the distinctiveness of the genus by pointing out the peculiar character of the spores. It also is the most advanced genus in the family with its complex venation and reduced laminar architecture being end products of its adaptive development. The principal characters of *Cnemidaria* rarely, if ever, occur in the other squamate genera.

The present study is part of a broad investigation of the neotropical Cyatheaceae (Tryon, 1970; Tryon & Tryon, 1982). The anatomy of representative species of *Sphaeropteris* and *Cnemidaria* is elucidated to provide data that can be used to determine the taxonomic status and phyletic position of these two genera. Previous workers (Holttum & Sen, 1961; Sen, 1964; Lucansky & White, 1974) have already shown the importance of anatomy and morphology to the study of this group of plants.

Materials and Methods

The following species were examined in this study: *Sphaeropteris elongata* (Hook.) Tryon, *S. senilis* (Kl.) Tryon, *Cnemidaria mutica* var. *mutica* (Maxon) Stolze and *C. mutica* var. *grandis* (Maxon) Stolze. Previous workers had considered these varieties of *C. mutica* to be distinct species (Maxon, 1912; Christensen, 1938; Tryon, 1970). Developing shoot tips were collected along roadsides or in montane and lowland rainforests in Costa Rica and Venezuela. Voucher specimens are on file in the herbarium of Duke University (DUKE).

Plant materials were killed and fixed in formalin-acetic acid-alcohol (FAA) and sectioned on a macrotome (Lucansky, 1976a). Sections (slices) were partitioned into manageable sizes, dehydrated in a tertiary-butyl alcohol series and embedded in paraffin (Johansen, 1940). Sections (8 μm) were made and stained with safranin-fast green. Stained sections were photographed with a 35 mm Nikon M35 S camera, and entire slices were photographed with a 35 mm single-lens reflex camera. Unless otherwise noted, the results given are based upon the combined data of all species.

Results and Discussion

Although members of *Sphaeropteris* are arborescent, species of *Cnemidaria* are characterized by an acaulescent habit. The caudices of some species of

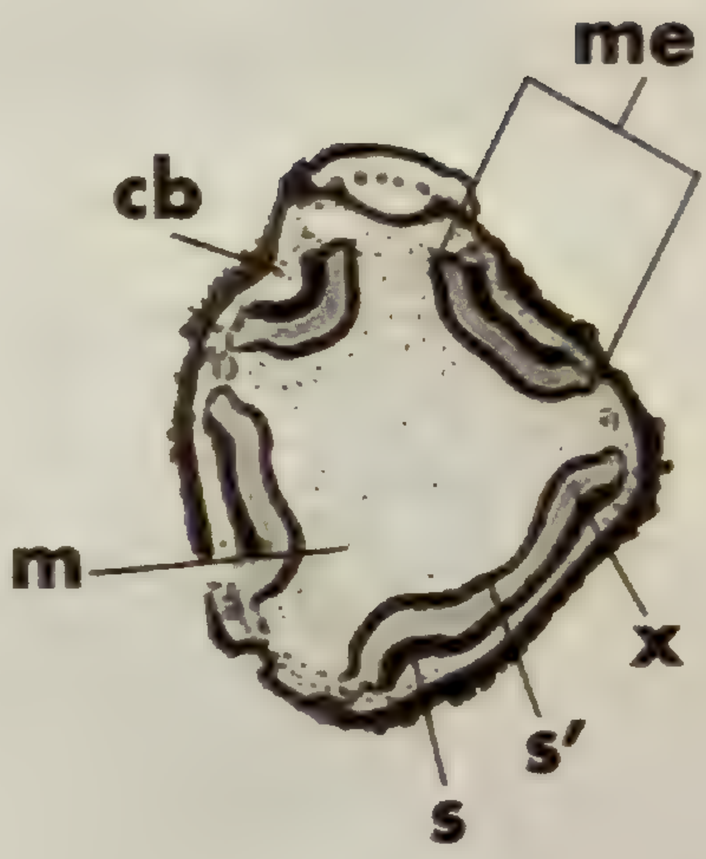
Cnemidaria, however, many reach 1.5 meter in length (Stolze, 1974). The stipe scales of *Sphaeropteris* are conform, whereas the scales of *Cnemidaria* are marginate. Certain species of *Sphaeropteris* may possess one or more dark apical setae on a scale, while the scales of *Cnemidaria* lack apical setae (Tryon, 1970, Tryon & Tryon 1982). The costae of *Sphaeropteris* (and other squamate genera) are pubescent adaxially, whereas trichomes, if present, occur only on the abaxial surface of the costae (and other axes) of *Cnemidaria*. A simple leaf architecture, costal areoles and a whitish arachnoid scurf also characterize *Cnemidaria*, but are lacking in *Sphaeropteris*. Spores of *Cnemidaria* with their three large equatorial pores are unique in the family Cyatheaceae (Stolze, 1974).

The stelar pattern in the mature stems of both *Sphaeropteris* and *Cnemidaria* is a dictyostele with overlapping leaf gaps and consists of individual vascular bundles (meristeles), each surrounded by sclerenchymatous tissue (Figs. 1–4). The number of meristeles varies from 3–5, depending upon the species and length of the leaf gaps.

A single-layered epidermis composed of variously shaped, thick-walled cells typically is sloughed in mature sporophytes, although remnants of this layer may persist. In *S. elongata* the epidermal cells are thin-walled and occasionally filled with tannins. Previous workers had reported that the outer walls of the epidermal cells may be thickened or cutinized (Sen & Mittra, 1966; Sen, 1968). The outermost layer of the stems of both genera studied is typically a hypodermis composed of two zones that are variable in thickness (Figs. 5, 6). The outer zone typically is partially sloughed and composed of variously shaped, thick-walled parenchyma cells filled with tannins and/or starch grains. The inner zone consists of sclerified, thick-walled parenchyma cells that closely resemble fibers (Fig. 5, 6). These cells are formed by secondary sclerosis of the walls with retention of the nucleus (Sen, 1968), and generally possess tannins and/or starch grains. The inner zone in *S. elongata* may be quite extensive and possess large, randomly-distributed mucilage-sac cells—singly or in group of 2–7 cells (Fig. 10). Distinctive cubical cells (to be discussed later) are noted between the hypodermis and the cortex in both species of *Cnemidaria* and *S. senilis* (Figs. 5). In *S. senilis* the hypodermis consists of only a single zone of sclerified parenchyma cells filled with tannins. Previous workers (Ogura, 1972; Mehra & Singh, 1955) also reported a single hypodermal zone of fibers, but such a homogenous layer may be due to the loss of the outer zone of parenchyma cells or the age (young) of the plant. A two-zoned hypodermis represents a unifying character in the group, and has been previously reported for the other genera (except *Metaxya*) in the family (Lucansky, 1976b, 1977, 1982).

Although Sen (1964) found that a band of sclerenchyma tissue may occur between the cortical layers in *Dicksonia* and *Culcita*, the cortex in the species in this study consists solely of large, thin-walled parenchyma cells filled with tannins and/or starch grains. Large mucilage-sac cells are randomly distributed in the cortex, singly or in a group of 2–10, and form an articulated laticiferlike system (Figs. 7, 8). Similar idioblasts have been reported for *Lophosoria* (Lucansky, 1982), the other squamate genera (Lucansky, 1976b, 1977) and *Dicksonia* (Williams, 1925). Schütze (1906) called these idioblasts excretion containers, rath-

1



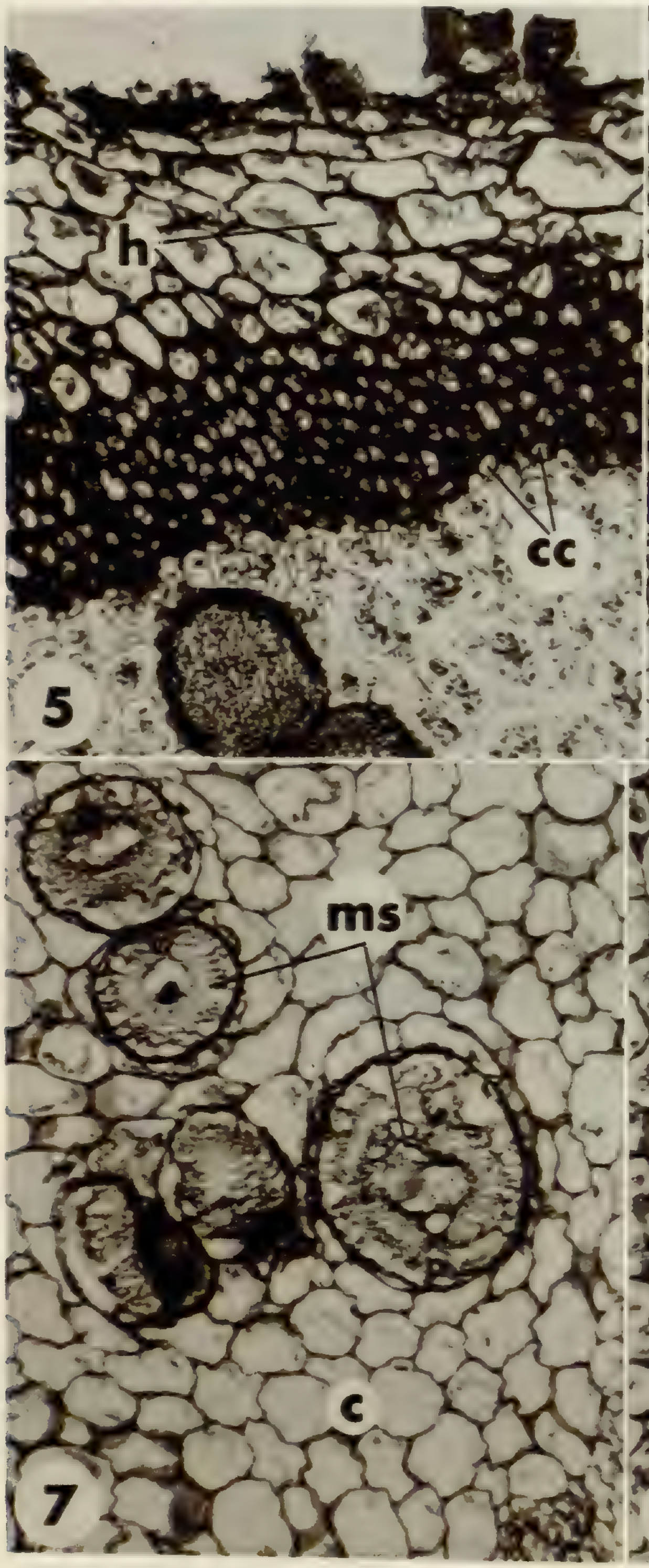
2

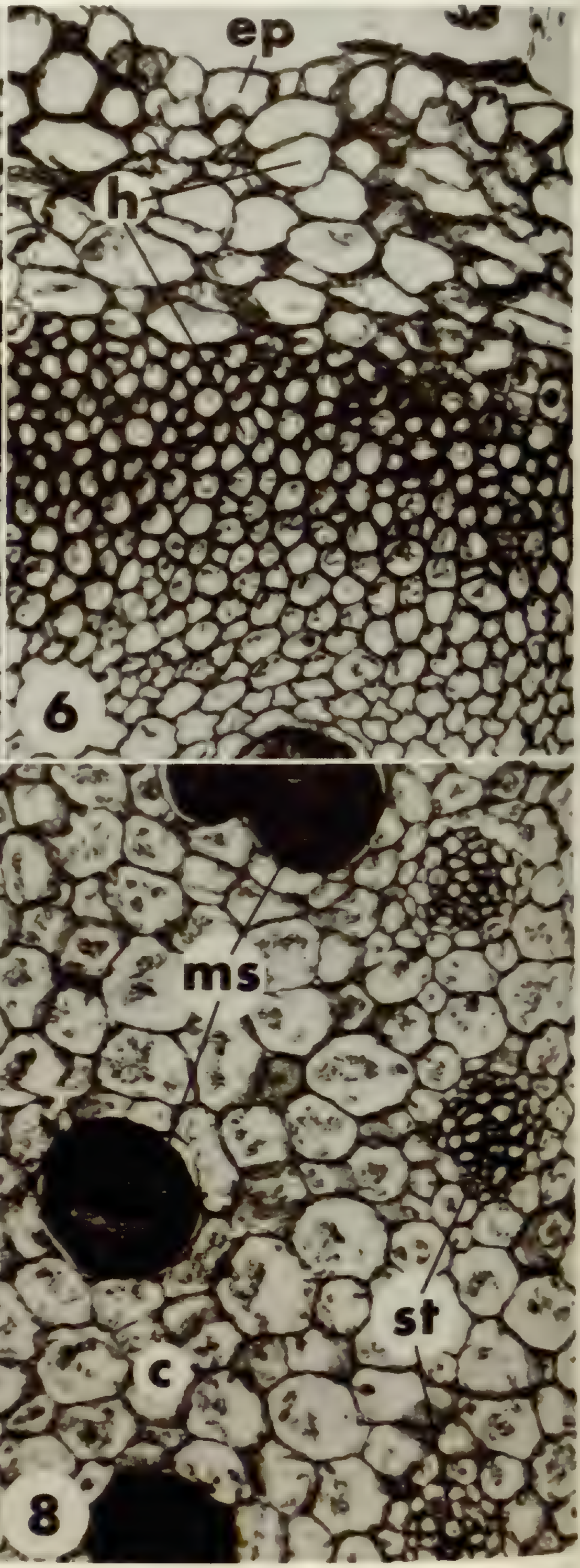
3

4

FIGS. 1–4. Transections of tree-fern stems. FIG. 1. *Sphaeropteris elongata*, ×8. FIG. 2. *Sphaeropteris senilis*, ×1.5. FIG. 3. *Cnemidaria mutica* var. *mutica*, ×1.7. FIG. 4. *Cnemidaria mutica* var. *grandis*, ×1.3. cb = cortical bundle, m = medullary bundle, me = meristele, s = external stelar sheath, s′ = internal stelar sheath, x = primary xylem.

er than secretion cells, and reported that they contain fatty acids or tannins, whereas Ogura (1972) found that they contain slime. Numerous small localized areas of sclerified parenchyma cells, filled with tannins, occur within the cortex of both species of *Cnemidaria* and *S. senilis* (Fig. 8), but are lacking in *S. elon-*



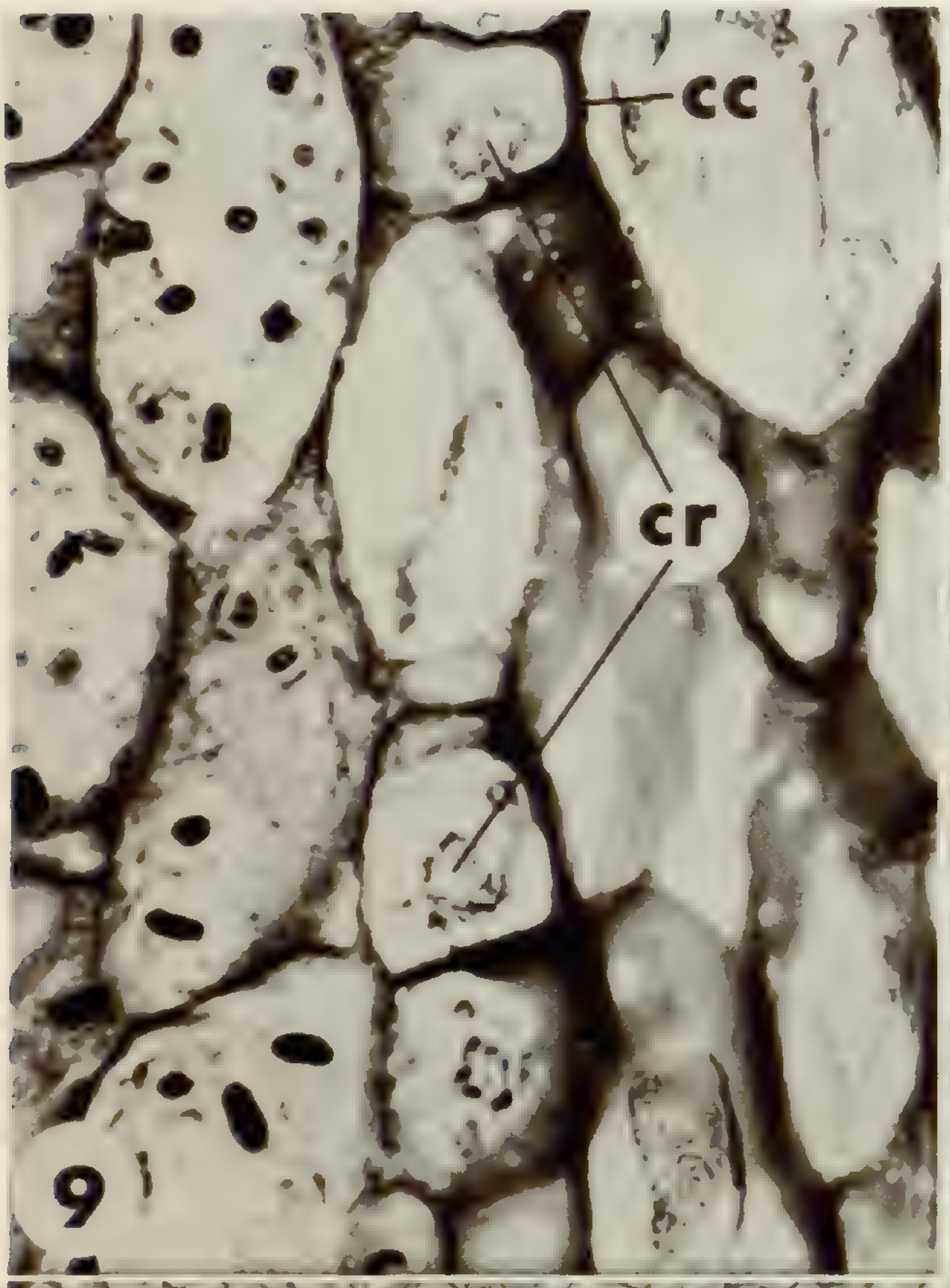


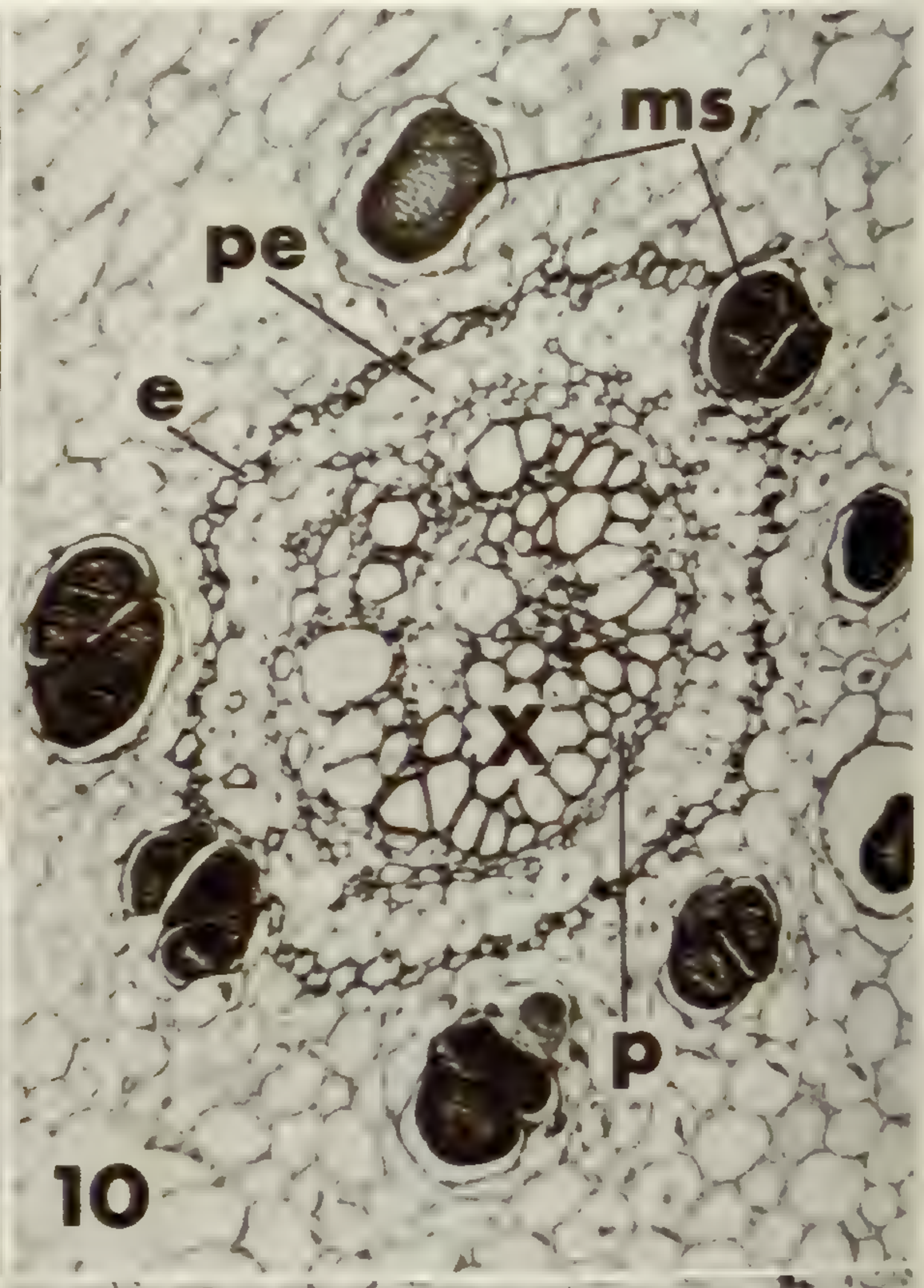
FIGS. 5–8. Anatomical details of tree-fern stems. FIG. 5. Two-zoned hypodermis of *Cnemidaria mutica* var. *grandis*, ×124. Note cubical cells. FIG. 6. Two-zoned hypodermis of *Sphaeropteris elongata*, ×130. FIG. 7. Mucilage-sac cells in cortical zone of *S. senilis*, ×126. FIG. 8. Localized areas of sclerified parenchyma in cortex of *C. mutica* var. *grandis*, ×144. c = cortex, cc = cubical cell, ep = epidermis, h, hypodermis, ms = mucilage-sac cell, st = sclerified (parenchyma) tissue.

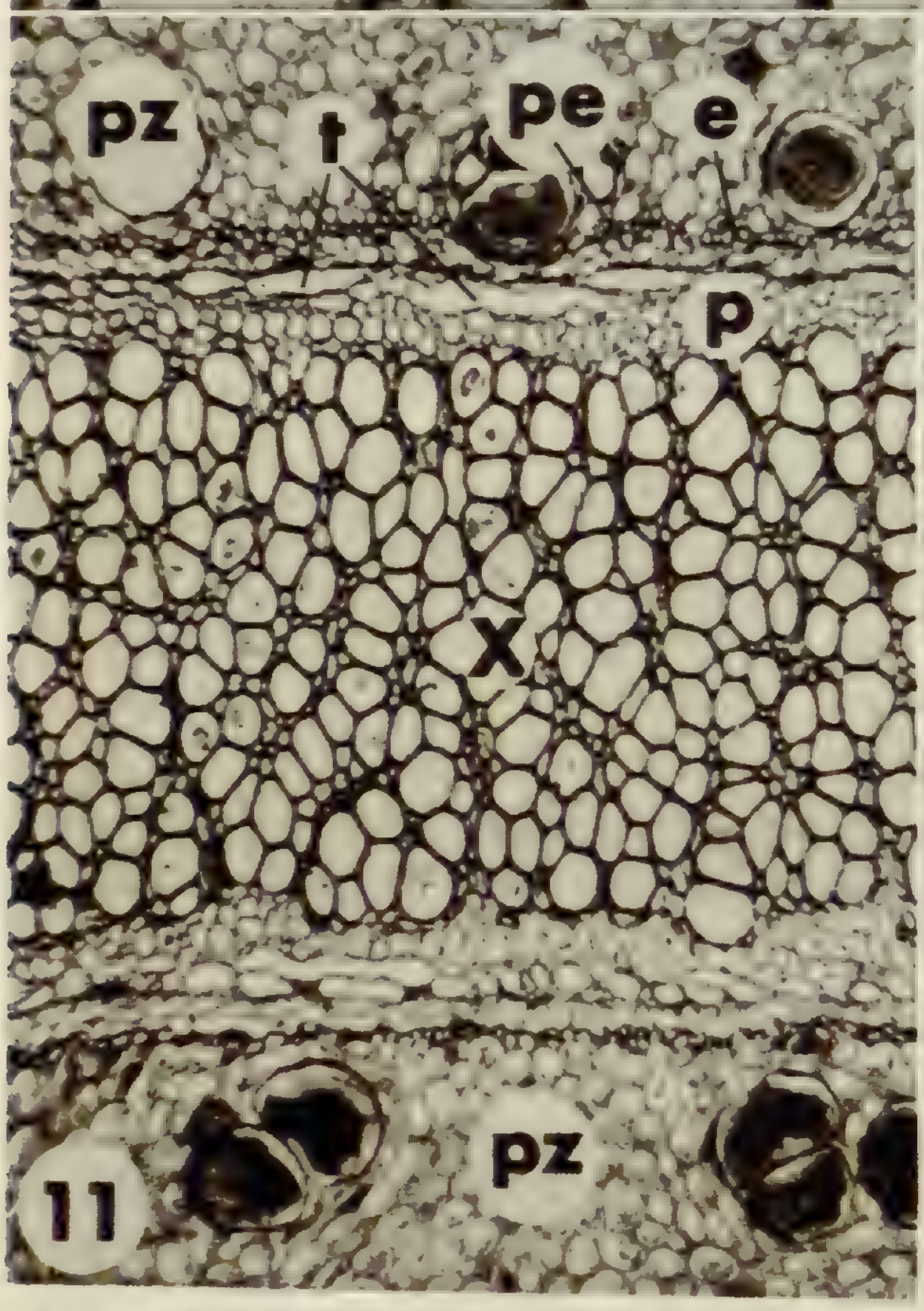
gata. Cubical cells occur between these sclerified areas and the cortex in these species (Fig. 9). Similar areas of sclerenchyma tissue have been noted in the cortex of *Cystodium* (Sen & Mittra, 1966), *Lophosoria* (Lucansky, 1982) and some species of *Trichipteris* and *Cyathea* (Lucansky, 1977), but do not occur in *Alsophila* and *Nephelea* (Lucansky, 1976b).

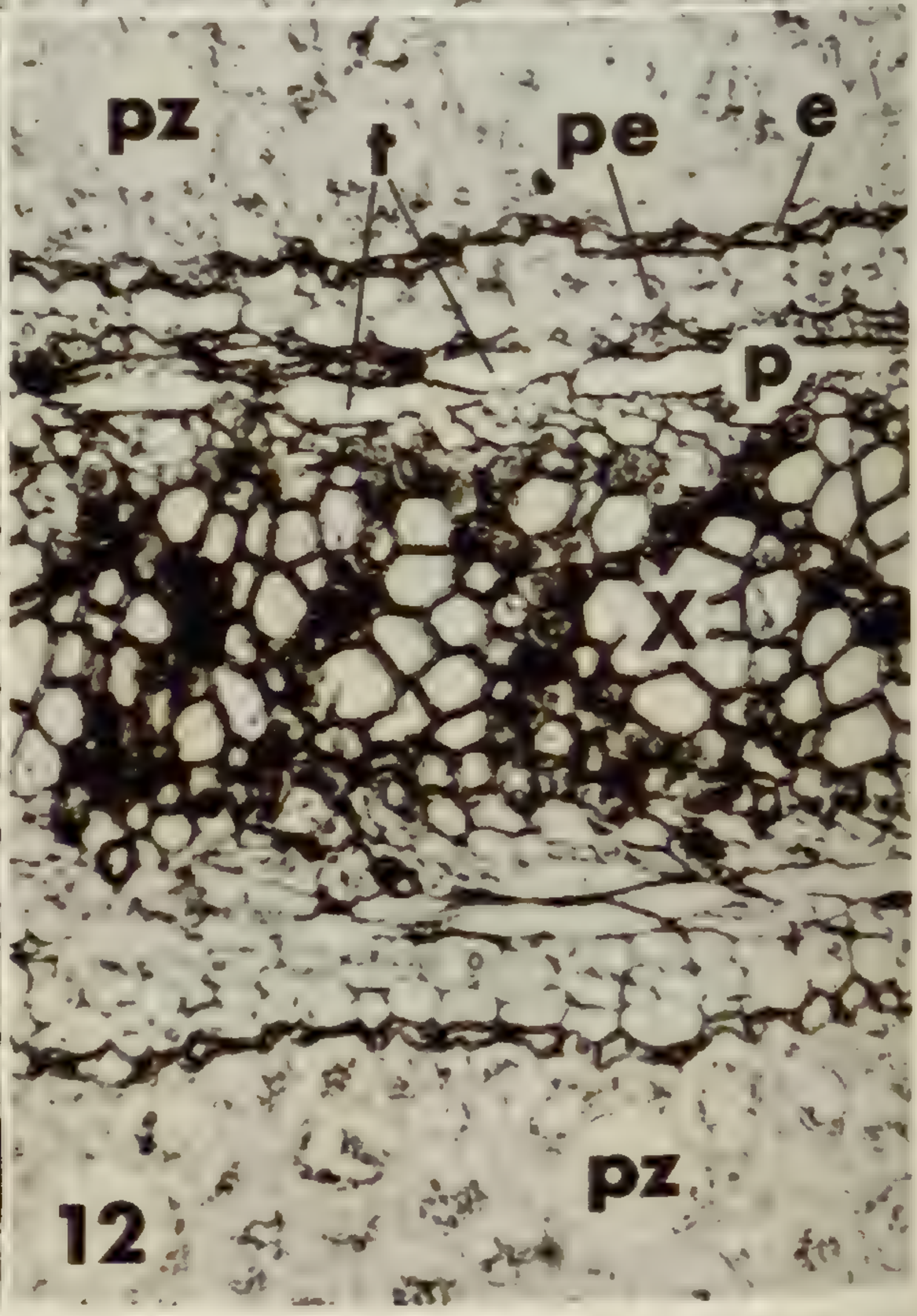
Cortical bundles are a characteristic feature of the Cyatheaceae (Lucansky, 1974b, Ogura, 1972), and are not found in any other group of ferns. Cortical bundles undergo divisions and fusions, end blindly in the cortex, fuse with meristeles and leaf traces, or infrequently proceed directly to the petiole as a leaf trace (Lucansky 1974b). Schütze (1906) believed that they are possibly involved with the movement and deposition of starch in the stem. In the present study cortical bundles occur in *S. elongata* and in *S. senilis* (Fig. 10), but are lacking in both species of *Cnemidaria*. These bundles may be large and form a conspicuous feature in *S. elongata* (Figs. 1, 10), but are small and relatively indistinct in *S. senilis* (Fig. 2). Cortical bundles have previously been found in some species of *Nephelea*, *Alsophila*, and *Trichipteris* (Lucansky 1976b, 1977), but are lacking in *Cyathea*, *Metaxya*, and *Lophosoria* (Lucansky 1977, 1982). Both Stolze (1974) and Tryon (1970) believe that *Cnemidaria* has probably arisen from a line of *Cyathea*, and the lack of cortical bundles in both genera supports this contention. These accessory bundles in *Sphaeropteris* may possess a partial sheath of sclerified parenchyma cells, although Schütze (1906) reported that cortical bundles usually lack a sheath. They are surrounded by a distinct single-layered endodermis that may be filled with tannins and possess Casparian strips (Fig. 10). A parenchymatous pericycle (1–3 layers) encircles the primary phloem, which consists of both sieve cells and phloem parenchyma. No tangential cells are found in the phloem of the cortical bundles, as previously reported for *Nephelea*, *Alsophila*, and *Trichipteris* (Lucansky 1976b, 1977). Small bundles have only xylary element in their centers, whereas large bundles may have one or more parenchymatous areas within the xylary mass. The primary xylem consists of tracheids with scalariform wall thickenings, with xylem parenchyma filled with tannins interspersed among these xylary elements (Fig. 10). Xylem maturation is mesarch.

In all species a meristele is surrounded by an external and internal stelar sheath composed of sclerified parenchyma (or sclerenchyma) cells filled with tannins. Both stelar sheaths arise from localized areas of sclerified cells that undergo fusion to form a continuous sheath (Lucansky & White, 1976). The presence of sclerified tissue around the individual meristeles is a characteristic feature of the Cyatheaceae, and had been previously noted in other cyatheoid genera (Lucansky 1976b, 1977, 1982; Ogura, 1972). Both stelar sheaths typically are delimited externally and internally by a single layer of cubical cells. These distinctive cells are greatly thickened on three walls (wall proximal to thin-walled parenchyma cells remains thin-walled), and each cell contains a single, large solitary crystal (Fig. 9). The crystalloid structure in each cell is insoluble in H_2SO_4 (Holttum & Sen, 1961) and is thought to be composed of silica (Sen, 1968). Cubical cells have been found in other genera of neotropical Cyatheaceae (Lucansky 1976b, 1977, 1982), and occur in the cortex of certain dicksonioid species









FIGS. 9–12. Anatomical details of tree-ferns stems. FIG. 9. Cubical cells with crystals in *Sphaeropteris senilis* between external stelar sheath and parenchymatous zone of a meristele, ×560. FIG. 10. Large cortical bundle in *S. elongata*, ×123. FIG. 11. Meristele of *S. elongata* showing tangential cells, ×57. FIG. 12. Meristele of *Cnemidaria mutica* var. *mutica* showing tangential cells, ×120. cc = cubical cell, cr = crystal, e = endodermis, ms = mucilage-sac cell, p = primary phloem, pe = pericycle, pz = parenchymatous zone, t = tangential cell, x = primary xylem.

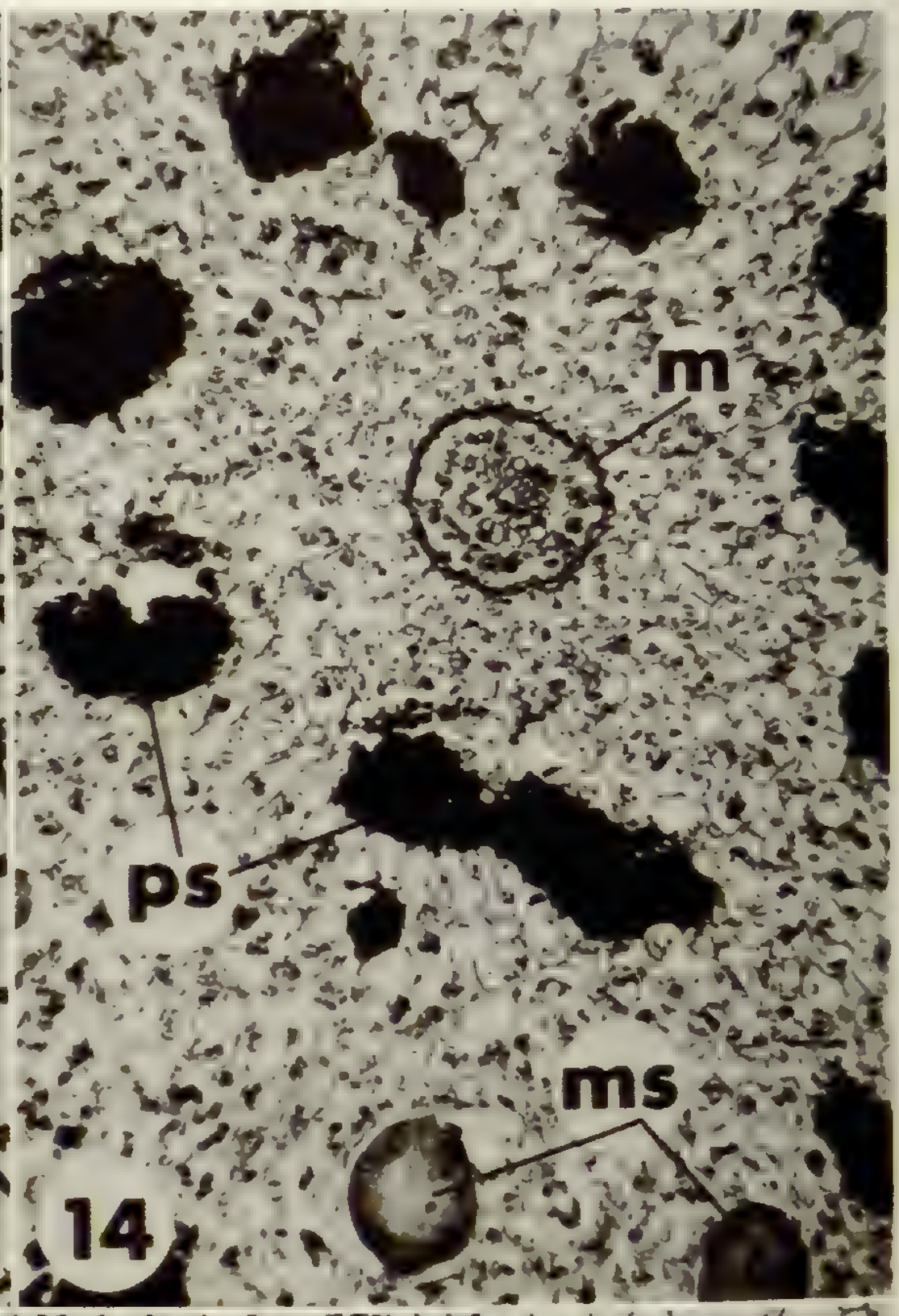
(Sen, 1964). Although Ogura (1972) thought that these cells were sclerenchyma cells, their living protoplast, wall morphology, position and resemblance to parenchyma cells in young stems indicate that they are thick-walled parenchyma. Sen (1964) also believed that they were not sclerenchyma cells based upon their rate of cell division and cellular inclusions.

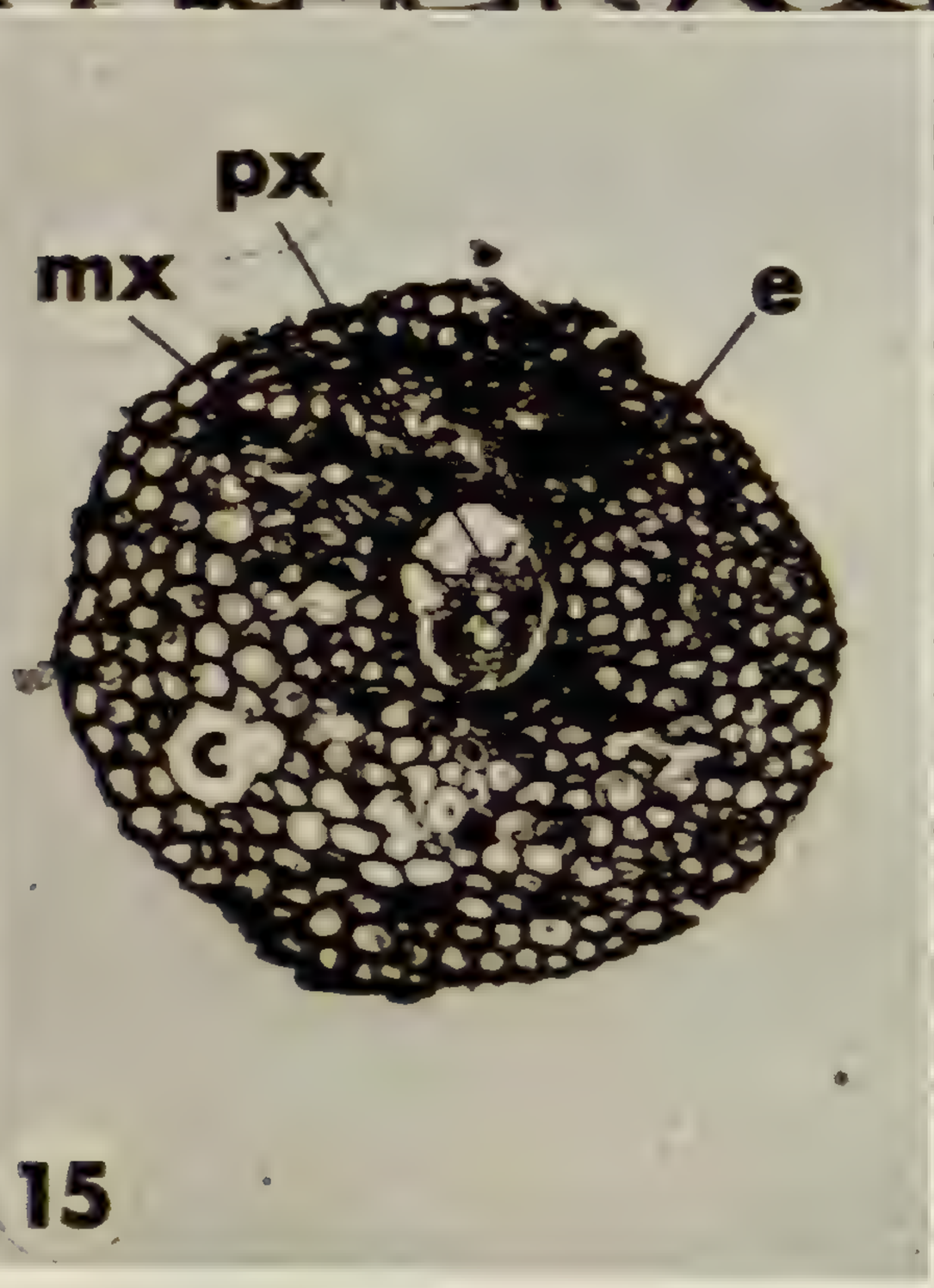
A parenchymatous zone composed of large, thin-walled parenchyma cells filled with starch grains and tannins separates the stelar sheaths from each meristele (Figs. 1, 12). Large mucilage-sac cells occur singly or in groups of 2–7 within this zone (Fig. 11). Schütze (1906) believed that these parenchymatous zones may function in the conduction and storage of carbohydrates.

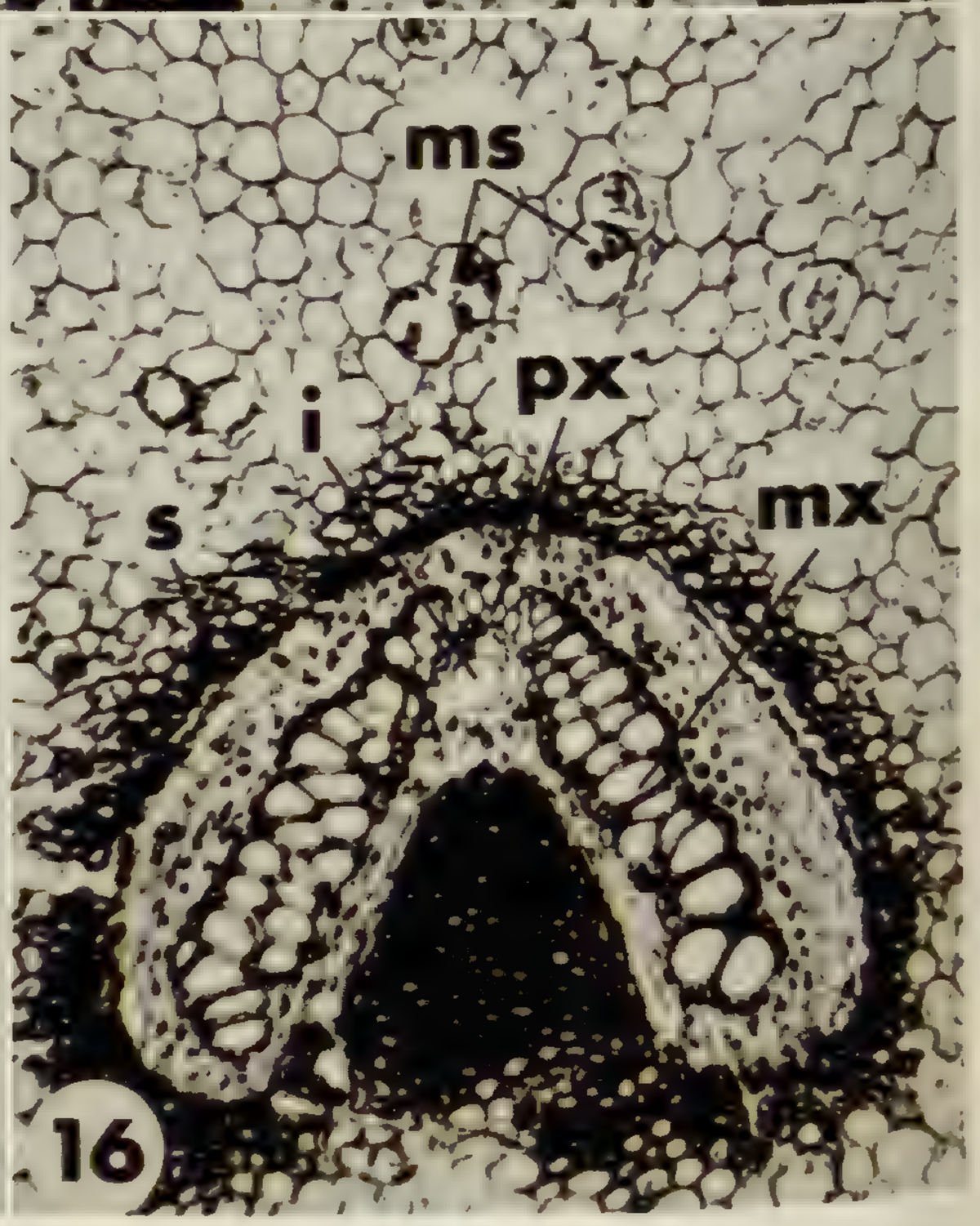
Each meristele in an amphicribral bundle delimited by a distinct endodermis filled with tanniferous substances (Figs. 11, 12). Distinct Casparian strips are lacking in the walls of these cells. A pericycle of 2–3 rows of thin-walled parenchyma cells completely encircles the primary phloem. Although Ogura (1927) reported that the protophloem was usually compressed, thick-walled, and swollen, the protophloem and metaphloem typically are indistinguishable in material studied. The latter tissue is composed of sieve cells, phloem parenchyma cells filled with tannins, and distinctive tangential cells (Figs. 11, 12). The phloic tangential cells are large and elongate tangentially in transection (Fig. 13) and form a characteristic feature of the Cyatheaceae. They are more numerous in *Cnemidaria* than in *Sphaeropteris*, and their position and pattern of arrangement is similar to that reported for the other cyatheoid genera (Lucansky 1976b, 1977, 1982). They represent specialized sieve cells that are devoid of nuclei, possess sieve areas on their lateral walls and accumulate callose (Sen, 1964), although they have been variously referred to as false sieve tubes (Schütze, 1906) or mucilage cells (Ogura, 1927, 1972). According to Ogura, these distinctive cells may be partially or entirely replaced by mucilage cells or longitudinally elongate cells.

The primary xylem is primarily composed of tracheids with scalariform wall thickenings, with xylem parenchyma cells filled with tannins and starch grains interspersed among these tracheary elements (Figs. 11, 12). Although earlier studies (Ogura, 1927; Sen, 1964) reported that protoxylem is usually absent in the primary xylem of mature stems, tracheids with spiral wall thickenings were infrequently seen in the present study. Each meristele is composed predominantly of metaxylem and xylem maturation is mesarch in all species studied.

The pith contains large, thin-walled parenchyma cells that frequently contain starch grains and tannins. Large mucilage-sac cells occur randomly in the pith, either singly or in groups of 2–5. In all species studied, numerous medullary bundles are scattered in the pith (Fig. 14) and represent another characteristic feature of the Cyatheaceae. They have been found in the other squamate genera in the family (Lucansky, 1974b, 1976b, 1977; Ogura, 1972), but are lacking in *Metaxya* and *Lophosoria* (Lucansky, 1974a, 1982). Adams (1977) reported that the medullary bundle system of *Cyathea fulva* consists of a central network and a peripheral one composed of three types of bundles. Medullary bundles arise *de novo* in the pith (Ogura, 1927, Lucansky, 1974b) or are separated from the leaf-gap margins (Godwin, 1932; Lucansky, 1976b). They undergo fusions and







FIGS. 13–16. Anatomical details of tree-ferns stems. FIG. 13. Tangential cells in the primary phloem of meristele of *Sphaeropteris elongata*, ×139. FIG. 14. Medullary bundle with partial sheath in the pith region of *Cnemidaria mutica* var. *mutica*, ×581. Note mucilage-sac cells. FIG. 15. Transection of diarch root of *S. senilis*, ×67. FIG. 16. Transection of petiole strand of *S. senilis*, ×71. Note intercellular space. c = cortex, e = endodermis, i = intercellular space, m = medullary bundle, ms = mucilage-sac cell, mx = metaxylem, p = primary phloem, pe = pericycle, ps = partial sheath, px = protoxylem, s = sheath, t = tangential cell, x = primary xylem.

divisions, end blindly in the pith, fuse with a meristele or leaf trace or proceed directly to the petiole as a leaf trace (Lucansky, 1974b; Lucansky & White, 1974; Adams, 1977). The medullary bundles are identical in cellular composition to cortical bundles. Although phloic tangential cells have been found in medullary bundles of certain species of *Alsophila* and *Nephelea* (Lucansky, 1976b), no phloic tangential cells occur in these bundles in the species studied. Small medullary bundles typically have only xylary elements in their centers, whereas larger bundles may have 1–several parenchymatous areas within the xylary mass. Each bundle may be partially or totally surrounded by a partial sheath composed of sclerified parenchyma cells (Fig. 14), although Ogura (1972) reported that medullary bundles usually lack a sheath. Only those bundles located along the internal stelar sheath normally lack such tissue in the species in this study. In addition to these sheaths, variously sized localized areas of sclerified parenchyma cells are randomly scattered in the pith of both *Sphaeropteris* and *Cnemidaria*. Cubical cells occur between these areas and the pith proper. Localized areas of sclerified tissue also occur infrequently in *Nephelea* and *Trichipteris*, but commonly occur in *Cyathea* (Lucansky 1976b, 1977).

Transections of the adventitious roots show similar anatomical features in all species studied (Fig. 15). The epidermis is typically sloughed in mature roots, and the outer cortex, composed of thick-walled parenchyma cells, forms the outer boundary of the organ. The epidermis, if present, is composed of thick-walled cells filled with tannins. The outer cortical cells also are frequently filled with tannins and may be partially sloughed. The inner cortex, composed of thicker-walled, sclerified parenchyma cells with tannins, typically forms the bulk of the cortex. Previous workers (Schütze, 1906; Sen, 1968) found the position of these two cortical zones reversed, while other investigators (Ogura, 1927, 1972; Lucansky 1976b, 1977, 1982) indicated a similar arrangement for the zones that comprise the cortex in the cyatheoid genera. In *S. senilis* the cortex may be composed of three zones of parenchyma cells, based upon wall thickness. A distinct endodermis composed of a single layer of cells filled with tanniferous substances delimits the stele (Fig. 15). A pericycle (1–2 cells) composed of large, thin-walled, tanniferous parenchyma cells surrounds the vascular tissue. The primary phloem consists of sieve cells and phloem parenchyma, while the primary xylem is composed primarily of scalariform pitted metaxylem and some spiral and reticulate—scalariform protoxylem. The xylem is diarch with exarch maturation (Fig. 15). Vascular parenchyma cells filled with tannins occur between the primary phloem and xylem.

Root traces originate either from a meristele or from the base of leaf traces and pass obliquely through the cortex. Leaf traces arise at successive levels in a leaf gap and proceed to the petiole to form a much-dissected vascular pattern that is similar for all species studied (Lucansky & White, 1974). Both types of traces are identical in cellular composition to the accessory bundles.

Transections of a petiole show similar anatomical characteristics in both *Sphaeropteris* and *Cnemidaria*. A single-layered epidermis composed of small thick-walled, tanniferous cells forms the outer boundary of a petiole. A two-zoned hypodermis occurs beneath the epidermis. The outer zone consists of

large, thick-walled parenchyma cells, whereas the inner zone is typically more extensive and composed of small, sclerified parenchyma cells. Both zones frequently contain tannins, and mucilage-sac cells infrequently occurs in the inner zone in *S. elongata*. Ground tissue, composed of thin-walled parenchyma cells with starch grains and/or tannins, comprises the bulk of a petiole. Large mucilage-sac cells are randomly scattered in this tissue in both genera—either singly or in groups of 2–3 (Figs. 16). Larger groups (4–10) of these idioblasts are infrequently found in *Cnemidaria*. Similar idioblasts were noted in the petioles of the other cyatheoid genera, but are lacking in *Metaxya* (Lucansky 1976b, 1977, 1982). In addition to mucilage-sac cells, small localized areas of sclerified parenchyma cells occasionally occur in the ground tissue of both species of *Cnemidaria*, but are absent in *Sphaeropteris*. These areas arise *de novo*, and are scattered in this tissue. A layer of cubical cells surrounds each area, and also occurs between the ground tissue and inner hypodermal zone in *C. grandis*.

Each petiole strand is surrounded partially or totally by a sheath of thick-walled, sclerified parenchyma cells (infrequently some cells become fibers) (Fig. 16). A layer of cubical cells may occur on both sides of a given sheath in *Cnemidaria*. Cellular composition and arrangement of the stele of each petiole strand is similar to a meristele or accessory bundle of the stem. Tangential cells infrequently occur in the primary phloem in both species of *Cnemidaria*, but are not found in *Sphaeropteris*. The primary xylem is U- or V-shaped, with the protoxylem found in a median position on the concave side of the vascular tissue (Fig. 16). Typically a single, large intercellular space is produced schizolysigenously adaxial to the protoxylem pole in both genera. Previous studies have reported that the protoxylem may partially disintegrate and form a cavity with tyloses (Schutze, 1906; Ogura, 1927), although no cavities were noted in the petioles of other cyatheoid genera (Lucansky 1976b, 1977, 1982).

Based upon the species studied, *Sphaeropteris* and *Cnemidaria* are similar in many anatomical characters, but are significantly different in certain morphological and anatomical features to warrant their generic separation in the Cyatheaceae. Anatomically they are closely related and form a natural group with the other squamate genera in the family, and should not be widely separated in any phyletic scheme proposed for the family.

Literature Cited

Adams, David C. 1977. Ciné analysis of the medullary bundle system in *Cyathea fulva*. Amer. Fern J. 67:73–80.

Bower, F. O. 1912. Studies in the phylogeny of the Filicales. II. *Lophosoria*, and its relationship to the Cyatheoideae and other ferns. Ann. Bot. (London) 26:269–323.

Christensen, C. 1938. Filicinae. In *Manual of Pteridology*, ed. F. Verdoorn. The Hague: Martinus Nijhoff.

Gastony, G. J. 1973. A revision of the fern genus *Nephelea*. Contr. Gray Herb. 203:81–148.

———. 1974. Spore morphology in the Cyatheaceae. I. The perine and sporangial capacity: general considerations. Amer. J. Bot. 61:672–680.

———. 1981. Spore morphology in the Dicksoniaceae. I. The genera *Cystodium*, *Thyrsopteris*, and *Culcita*. Amer. J. Bot. 68:808–819.

——— and R. M. Tryon. 1976. Spore morphology in the Cyatheaceae. II. The genera *Lophsoria*, *Metaxya*, *Sphaeropteris*, *Alsophila*, and *Nephelea*. Amer. J. Bot. 63:738–758.

GODWIN, H. 1932. Anatomy of the stele of *Cyathea medullaris* Sw. New Phytol. 31:254–264.
HOLTTUM, R. E. 1963. Cyatheaceae. In *Flora Malesiana*, Series II, Pteriodophyta, Vol. 1 (2):65–176.
———. 1964. The tree ferns of the genus *Cyathea* in Australasia and the Pacific. Blumea 12:241–274.
———. 1965. Tree-ferns of the genus *Cyathea* in Asia (excluding Malesia). Kew Bull. 19:463–487.
———. 1982. Species of *Cyathea* in the Western Pacific related to *C. multiflora* Sm. and allies in America. Kew Bull. 37:383–388.
——— and U. SEN. 1961. Morphology and classification of the tree ferns. Phytomorphology 11: 406–420.
——— and P. J. Edwards. 1983. The tree-ferns of Mount Roraima and neighboring areas of the Guayana Highlands with comments on the family Cyatheaceae. Kew Bull. 38:155–188.
JOHANSEN, D. A. 1940. *Plant microtechnique*. New York: McGraw Hill.
LUCANSKY, T. W. 1974a. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. I. *Metaxya* and *Lophosoria*. Amer. J. Bot. 61:464–471.
———. 1974b. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. II. The squamate genera. Amer. J. Bot. 61:472–480.
———. 1976a. The macrotome: a new approach for the sectioning of large plant specimens. Stain Technol. 51:199–201.
———. 1976b. Anatomical studies of the neotropical Cyatheaceae. I. *Alsophila* and *Nephelea*. Amer. Fern J. 66:93–101.
———. 1977. Anatomical studies of *Cyathea* and *Trichipteris* (Cyatheaceae). Amer. J. Bot. 64: 253–259.
———. 1982. Anatomical studies of the neotropical Cyatheaceae. II. *Metaxya* and *Lophosoria*. Amer. Fern J. 72:19–28.
——— and R. A. White. 1974. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. III. Nodal and petiole patterns; summary and conclusions. Amer. J. Bot. 61:818–828.
——— and R. A. WHITE. 1976. Comparative ontogenetic studies in young sporophytes of tree ferns. I. A primitive and an advanced taxon. Amer. J. Bot. 63:463–472.
MAXON, W. R. 1912. The tree ferns of North America. Smithsonian Inst. Annual Rep. 1911:473–491.
MEHRA, P. N. and J. P. SINGH. 1955. Observations of the anatomy of *Alsophila glabra* Hook. Sci. & Cult. 21:273.
OGURA, Y. 1927. Comparative anatomy of the Japanese Cyatheaceae. J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 1:41–350.
———. 1972. Comparative anatomy of vegetative organs of the pteridophytes. 2nd ed. in K. Linsbauer's *Handbuch der Pflanzenanatomie*. Bd. 7. T. 3. Berlin: Gebrüder Borntraeger.
SCHÜTZE, W. 1906. Zur physiologischen Anatomie einiger tropischer Farne, besonders der Baumfarne. Beitr. Wiss. Bot. (Stuttgart) 5:329–376.
SEN, U. 1964. Importance of anatomy in the phylogeny of tree ferns and their allies. Bull. Bot. Soc. Bengal 18:26–34.
———. 1968. Anatomy of *Culcita macrocarpa*. Canad. J. Bot. 46:43–46.
——— and D. MITTRA. 1966. The anatomy of *Cystodium*. Amer. Fern J. 56:97–101.
STOLZE, R. G. 1974. A taxonomic revision of the genus *Cnemidaria* (Cyatheaceae). Fieldiana, Bot. 37:1–98.
TRYON, R. M. 1970. The classification of the Cyatheaceae. Contr. Gray Herb. 200:1–53.
——— and A. F. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.
WILLIAMS, S. 1925. Some points on the anatomy of *Dicksonia*. Proc. Roy. Soc. Edinburgh. 45:286–296.

American Fern Journal 75(3):92–102 (1985)

Germination and Morphology of Spores of *Trichipteris corcovadensis*

LUCIANO M. ESTEVES and GIL M. FELIPPE
Departamento de Fisiologia Vegetal, Instituto de Biologia, C. P. 6109,
Universidade Estadual de Campinas, 13100 Campinas, Brasil
THEREZINHA S. MELHEM
Seção de Dicotiledôneas, Instituto de Botânica, C. P. 4005, 01000 São Paulo, Brasil

Several papers have dealt with the physiology of germination of pteridophyte spores. Published data show that spores of the majority of fern spores are light-sensitive. They also germinate in a wide range of temperatures, from 1°C (*Pteridium aquilinum*) to 35°C in the case of *Onoclea sensibilis* (Miller, 1968).

To our knowledge, no research has been published in Brazil on the physiology of ferns, with the exception of the germination of spores of *Cyathea delgadii* (Marcondes-Ferreira & Felippe, 1984). In this paper a study was made of germination of *Trichipteris corcovadensis* (Raddi) Copel. This species is a tree fern that occurs in the Serra do Mar area of Southern Brazil (Tryon, 1970), at elevations from 250 to 2100 meters (Barrington, 1978). A study of the spore morphology of several specimens was also carried out. Spore morphology of the species has been studied previously as *Trichopteris corcovadensis* (Kremp & Kawasaki, 1969; Erdtman & Sorsa, 1971).

MATERIALS AND METHODS

Material of *Trichipteris corcovadensis* was collected in the Reserva Biológica do Parque Estadual das Fontes do Ipiranga, São Paulo, Brazil, and identified by Dr. Paulo Windish (UNESP, São José do Rio Preto). Spores were collected from five different specimens gathered in March of different years. These collections were designated: A (1982 and 1983); B (1980, 1981, and 1982); C (1982 and 1983); D (1982); and E (1982). The spores were stored in closed bottles in the dark at 4°C. Some material from specimen B was also stored at 25°C. Spores from the five specimens were always stored separately, and the experiments were carried out with all five specimens for comparison.

Germination.—Germination studies were carried out using 25 ml of Mohr's solution, as modified by Dyer (1979), in an Erlenmeyer flask. The flasks with medium were autoclaved at 120°C for 20 minutes, and nystatin (50 units·ml^{-1}) was then added as recommended by Dyer (1979). Spores were scattered on the surface of the medium with the aid of a spatula. For each treatment, three flasks were used, and three microscope slides were prepared from aliquots from each flask. Five fields from each slide were examined through a microscope. Germination was considered as the protrusion of the rhizoid.

The experiments were carried out in growth cabinets (Forma Scientific model

24). Temperature was maintained at 25°C and light irradiance at 320 $\mu W \cdot cm^{-2}$ (continuous white fluorescent light). In some experiments the effect of constant temperature was examined (5, 10, 15, 20, 25, 30, 35, 40, and 45°C) as well as alternate temperatures of 5–25, 10–25, 15–25, 20–25, 30–25, 35–25, 40–25, and 45–25 (12 hours at each temperature in a 24 hour cycle, starting temperature 25°C). The effect of photoperiod was also studied and photoperiods of 1, 8, 12, 16 and 24 hours were used. The effect of irradiance was studied using light irradiances of 220, 320, 900, 1400, 1900, and 2500 $\mu W \cdot cm^{-2}$. The growth regulators gibberellic acid (GA_3), indole-3-acetic acid (IAA), 6-benzyladenine (6-BA) and 2-chloro-ethylphosfonic acid (CEPA) were used at the following concentrations: 0, 5, 25, 50, and 100 $\mu g \cdot ml^{-1}$ (the growth regulators were added to the flasks after autoclaving).

Viability of the spores was tested with acetocarmine according to Manton (1950). Spores were placed on a microscope slide with a drop of acetocarmine, covered with a cover slip, and pressed to break the spore walls (acetocarmine did not penetrate walls of intact spores). Spores with broken walls were counted 36 hours later, whether stained or not (data are presented as percentage of stained spores in relation to total spores with broken walls; 15 replicates were used).

Germination data are presented as the angular value: arc sin $\sqrt{\%}$. The percentage value is the number of germinated spores in relation to the total number of spores in each field. Statistical analysis was performed with data transformed into angular values. Where necessary the confidence interval (at 95%) and coefficient of variation are presented. Analysis of variance was also performed, and when $F_{5\%}$ was significant the $LSD_{5\%}$ was determined by Tukey's method as modified by Snedecor (1962). Lower case letters in tables compare the values within columns, taking into account the $LSD_{5\%}$.

Lipids and protein.—Total lipids were determined according to Gemmrich (1977), using 50 mg of spores in each extraction. Each specimen was analyzed in triplicate. Data are presented as mg lipid·100 mg^{-1} of spores. Soluble protein was determined according the dye-binding method of Bradford (1976), using 200 mg of spores in each determination and three replicates per treatment. Data are presented as mg protein·100 mg^{-1} spores.

Morphology of spores.—The spores were submitted to acetolysis according to Erdtman (1969). To observe the perine, spores were treated with sodium carbonate at 3% before acetolysis (Morbelli, 1974). The Wodehouse (1935) method was used to determine the presence of cellular content in the spores.

At least five slides were prepared for each sample. Measurements were made with 25 acetolysed spores, distributed on at least three slides (Salgado-Labouriau et al., 1965). Spores were measured not later than seven days from the preparation of the slide, to avoid swelling of spores (Melhem & Matos, 1972). The percentage of different spore shapes was determined in 45 fields, distributed on at least three slides (the same for the observation of the perine). To obtain the photomicrographs, an Olympus Vanox photomicroscope with an automatic camera was used. Kodak Panatomic-X, was used with a green filter to obtain details of the spores.

For specific details, the scanning microscope was also used, as described by

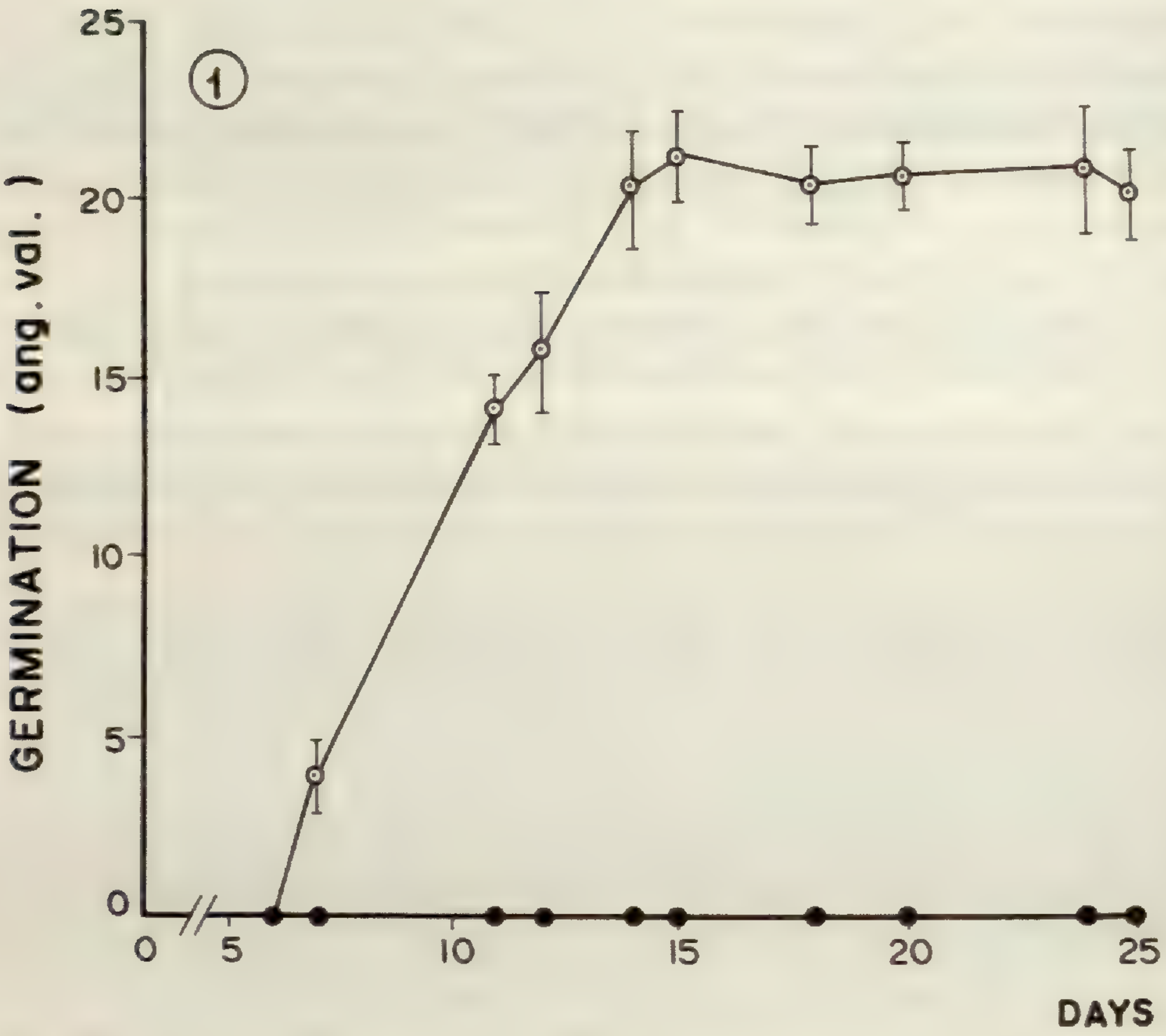


FIG. 1. Germination of spores of specimen A of *Trichipteris corcovadensis* at 25°C in constant white fluorescent light (320 $\mu W \cdot cm^2$) and darkness over a 25 day period. Open circles: light; closed circles: darkness. Confidence intervals (5%) are shown.

Cruz & Melhem (1984). Photographs were obtained from a scanning electron microscope GO, model JSM P15, with Kodak PX-135 (ASA 100 film).

The measurements presented were subjected to an analysis of variance, and when $F_{5\%}$ was significant, the $LSD_{5\%}$ (Tukey) was determined (Snedecor, 1962).

TABLE 1. Effect of Temperature on the Germination of Spores of *T. corcovadensis* (Specimen A) Kept in Continuous White Light.

Temperature* (°C)	Germination (angular value)
20	3.0[a]**
25	16.6[b]
30	10.4[c]
$LSD_{5\%}$ (Tukey)	1.8
25–20	12.7[a]
25–25	16.6[b]
25–30	13.0[a]
$LSD_{5\%}$ (Tukey)	2.4

* No germination occurred at 5, 10, 15, 35, 40, 45°C and in the alternate pairs 25–5, 25–10, 25–15, 25–35, 25–40 and 25–45°C.

** See Materials and Methods; a, b, and c, compare the values within columns.

TABLE 2. Effect of Light Irradiance and Photoperiod on the Germination of Spores of *T. corcovadensis* (Specimen A) Kept at 25°C.

Irradiance ($\mu W \cdot cm^{-2}$)	Germination (angular value)	Photoperiod (h)	Germination (angular value)
220	18.0[a]	0	0.0
320	18.9[a]	1	0.0
900	13.8[b]	8	17.6
1400	13.4[b]	12	18.5
1900	12.9[b]	16	17.7
2500	13.6[b]	24	18.9
$LSD_{5\%}$ (Tukey)	3.0		

$F_{5\%}$ not significant for photoperiods of 8, 12, 16, and 24 h.

RESULTS AND DISCUSSION

In all experiments dealing with germination, spores were obtained only from specimen A.

Figure 1 shows the results of one experiment in which spores were kept at 25°C either in continuous white fluorescent light or in darkness. No germination occurred in darkness and this was observed in all experiments. In the light, germination started on day 6. This proceeded at a fast rate until day 14 when a plateau was reached and maintained up to day 25. The maximum germination value was around 20 (angular value). In subsequent experiments germination was always counted 14 days after the start of the experiment.

No germination occurred at 5, 10, 15, 35, 40, and 45°C, even when these temperatures were alternated daily with 12 hours at 25°C. Germination occurred at 20, 25, and 30°C and with the alternate pairs 20–25 and 25–30°C (Table 1). At all temperatures tested, the spores germinated only in the light. Germination was higher with the two lower light irradiances used (Table 2) and germination occurred only (with the photoperiods tested) with photoperiods of 8 h and above (Table 2). In all the other experiments mentioned in this paper the light irradiance used was 320 $\mu W \cdot cm^{-2}$ and light was continuous (including the experiments in which temperature was tested shown previously).

TABLE 3. Effect of Growth Regulators on the Germination of Spores of Specimen A of *Trichipteris corcovadensis*, in Continuous Fluorescent White Light, at 25°C.

Concentration ($\mu g \cdot ml^{-1}$)	Germination (angular value)			
	IAA	GA_3	6-BA	CEPA
0	18.8[a]	18.5[a]	16.9[a]*	17.9
5	14.1[b]	17.2[a]	9.7[b]*	0.0
25	3.6[b]	10.2[b]	0.0	—
50	0.0	11.7[b]	—	—
100	—	3.3[c]	—	—
$LSD_{5\%}$ (Tukey)	2.2	1.8	—	—

* Student test.

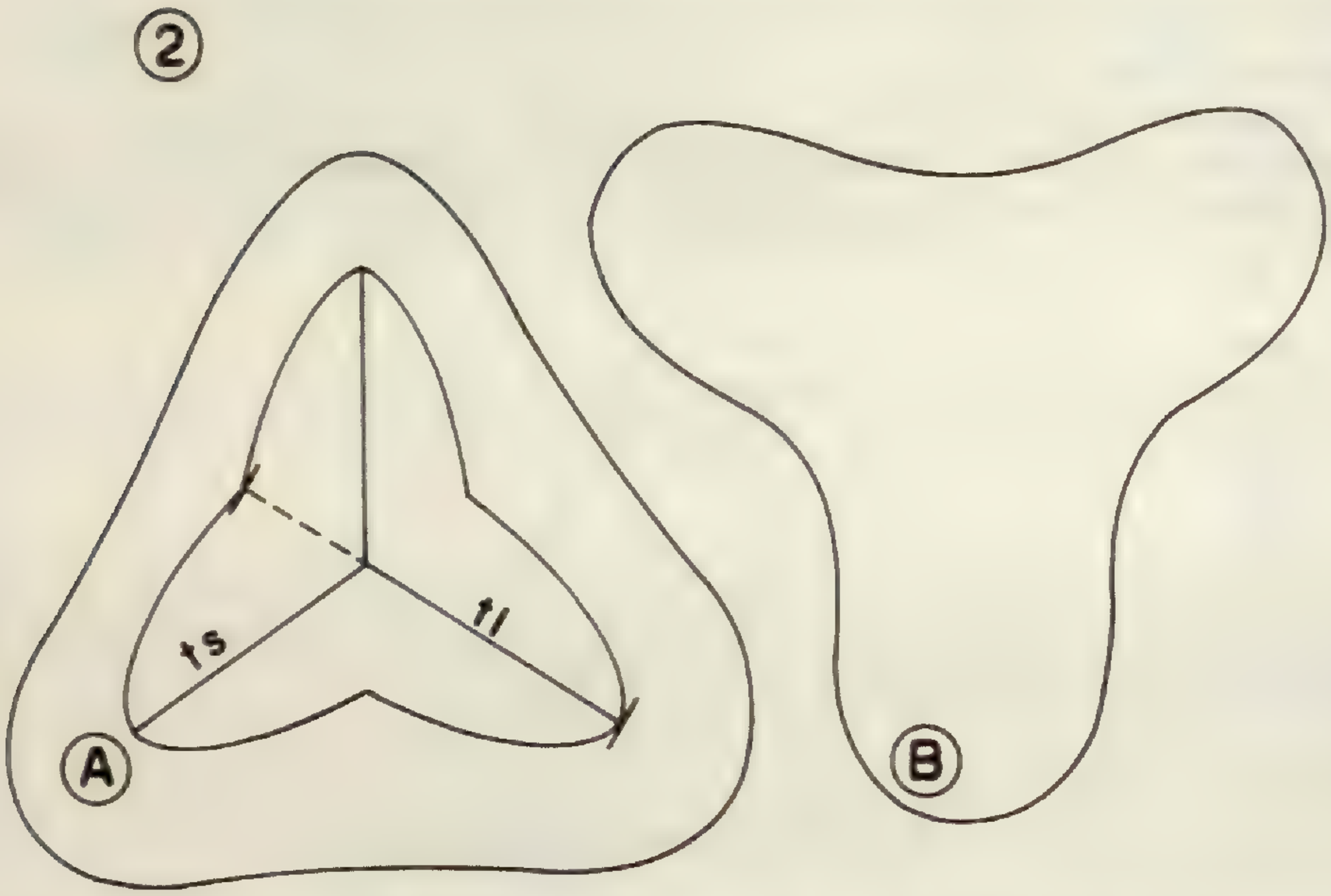


FIG. 2. Schematic representation of spores of *Trichipteris corcovadensis* showing the two extreme shapes. A = shape I; tl = trilete length; ts = trilete scar. B = shape II.

Spores of *T. corcovadensis* are positively photoblastic like the majority of pteriodophytes: Miller (1968) in his review showed that in 75 species studied only 4 germinated in darkness. *Cyathea delgadii* occurs with *T. corcovadensis* in the same region, shows germination over a wider range of temperatures (15 to 30°C), and is also a photoblastic positive species (Marcondes-Ferreira & Felippe, 1984).

All growth regulators tested had an inhibitory effect upon germination (Table 3) and were also unable to change the photoblasticity of the spores.

In all experiments the maximum values for germination were always low. This can also be seen in Table 4, in which the effect of period of storage at 4°C on germination is shown. After 135 days, germination is reduced. This was valid for spores of specimen A which were collected both in 1982 and 1983.

TABLE 4. Effect of Storage on the Germination of Spores of *Trichipteris corcovadensis* (Specimen A) in Continuous Fluorescent White Light at 25°C.

Storage at 4°C (days)	Germination (angular value)
15	19.1[a]
67	17.0[a]
92	17.1[a]
96	18.9[a]
102	18.4[a]
108	19.2[a]
134	16.9[a]
135	18.7[a]
238	11.5[b]
239	9.0[b]
254	9.2[b]
$LSD_{5\%}$ (Tukey)	2.9

TABLE 5. Shape Variation of Spores of Five Specimens of *Trichipteris corcovadensis* Collected in Different Years.

Specimen—year	Shape I	Shape II	Intermediate shapes
A—1982	100.0	0.0	0.0
B—1980	98.1	0.0	1.9
B—1981	96.4	0.0	3.6
B—1982	28.6	27.1	44.3
C—1982	30.5	22.9	44.6
D—1982	28.5	14.3	57.2
E—1982	31.5	22.4	46.1

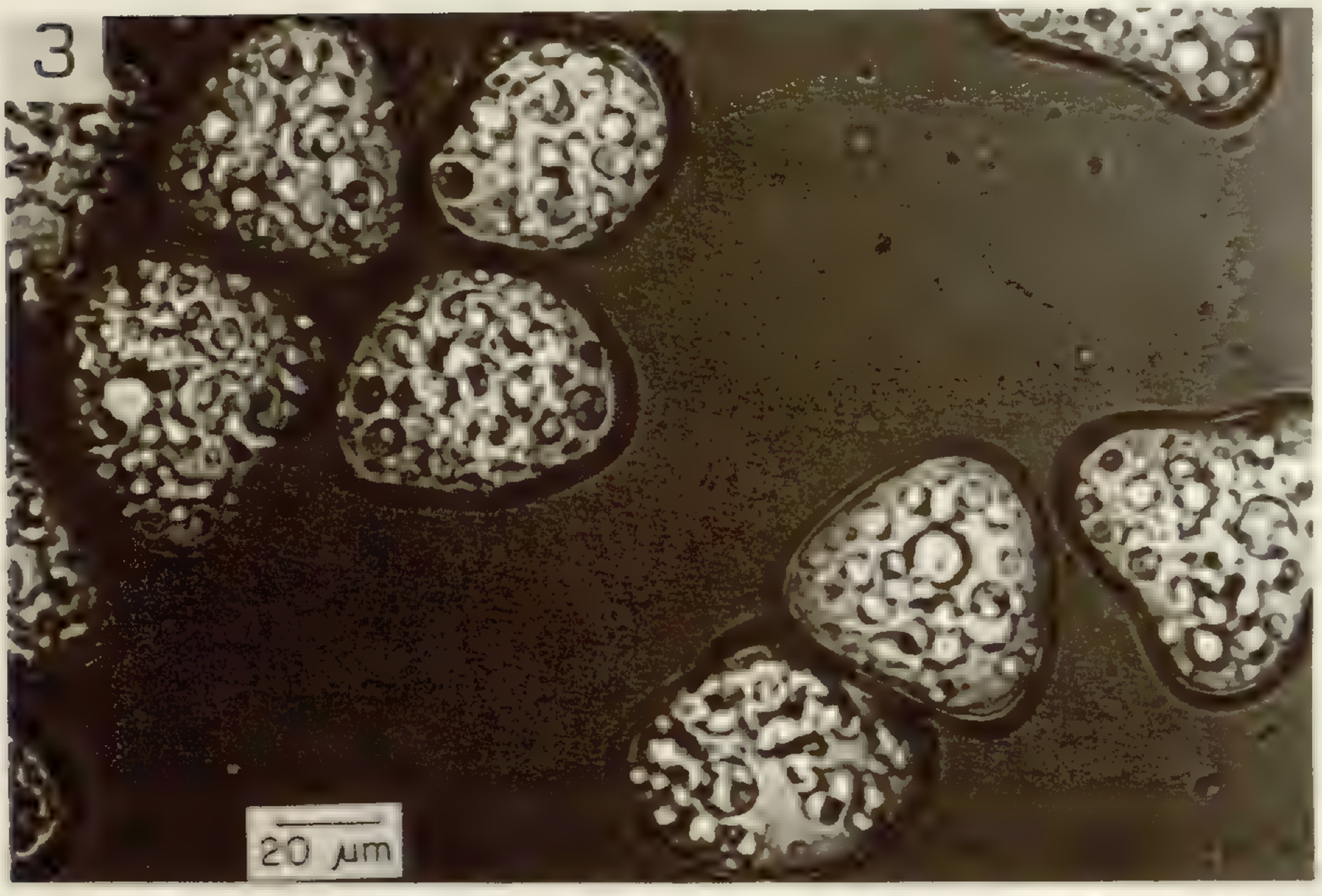
Spores collected from specimens B, C, D, and E never germinated. No treatment was able to change this. This lack of ability to germinate was observed with both freshly collected spores and stored spores. For example, spores of specimen B were stored at 4°C and 25°C and germination was tested from 15 to 960 days in regular intervals, but germination never occurred.

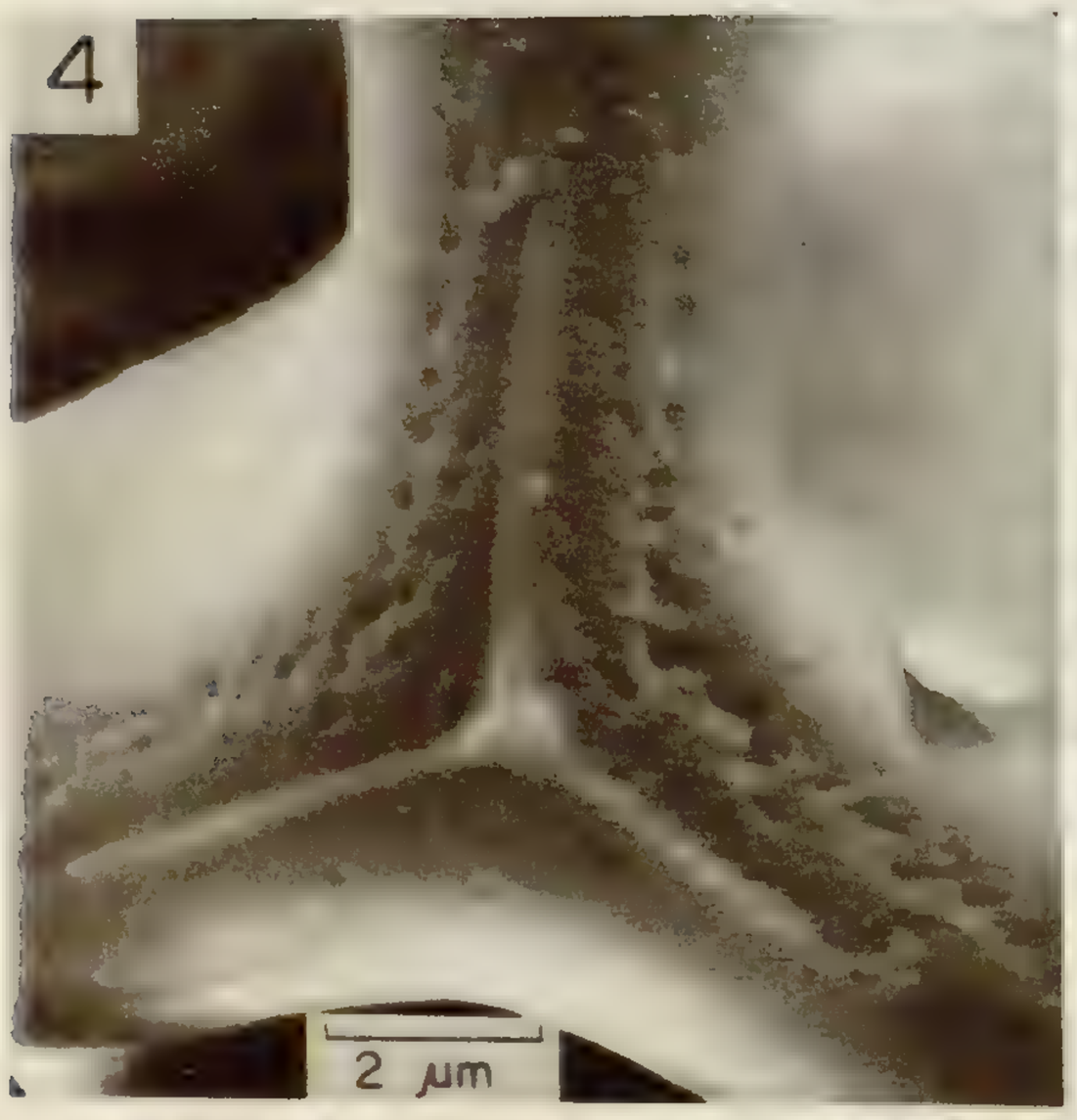
After acetolysis two extreme spore shapes could be seen: I (Fig. 2A) and II (Fig. 2B) and intermediate shapes between I and II. All spores of specimen A belonged to shape I (Table 5). Spores of specimen B collected in 1980 and 1981 belonged to shape I (around 95%) and intermediate shapes; specimens C, D, and E presented shapes I, II, and intermediates.

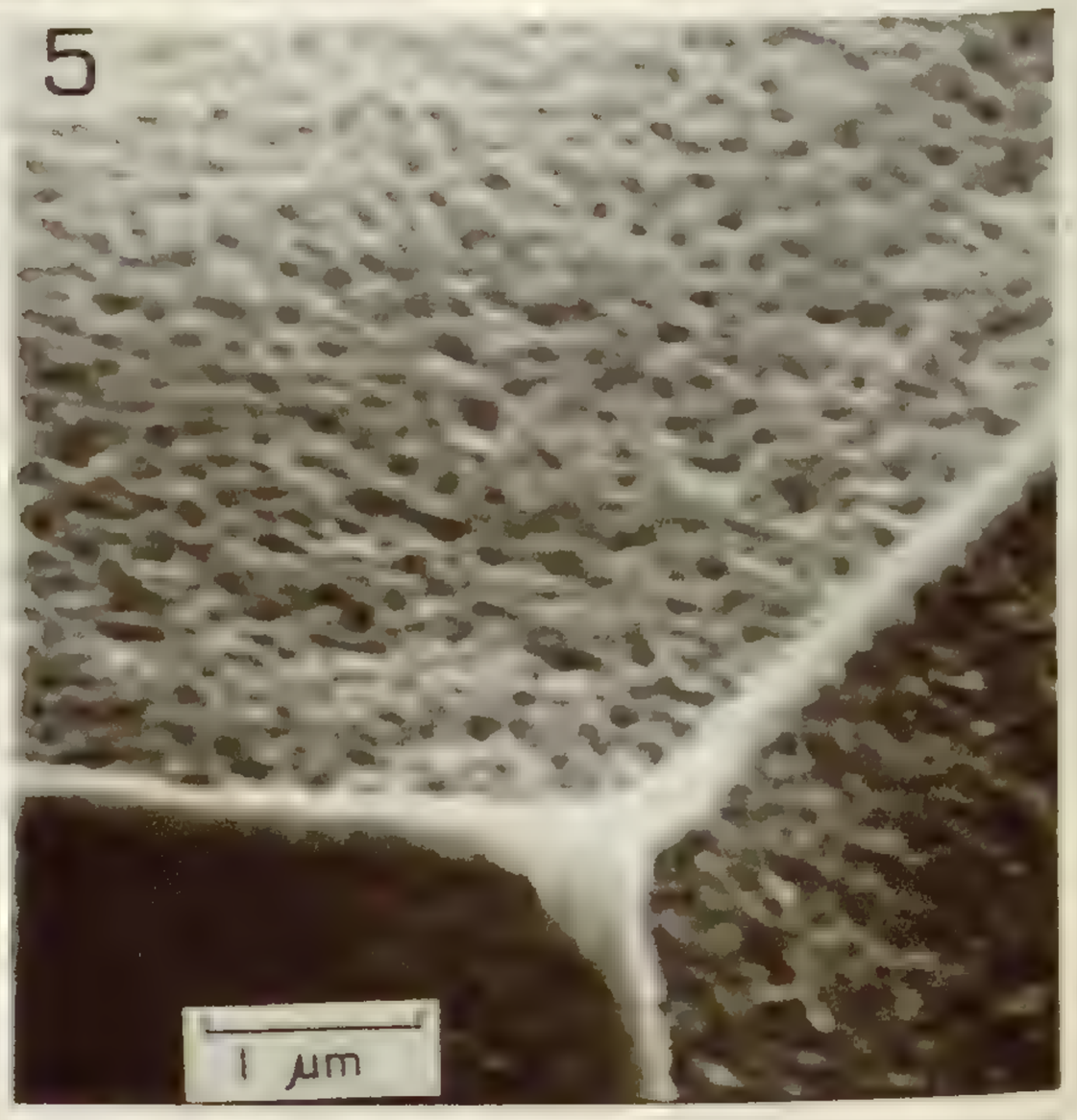
Viability was studied by staining the spores with acetocarmine. Acetocarmine stains the protoplasm and in so doing suggests that the spore is living or viable, but it does not signify that the stained spore can germinate. In this paper the term viable means living as defined by Bewley & Black (1978). For example, immature embryos of *Annona crassiflora* are viable or living, but they do not germinate for at least 200 days after harvest (Rizzini, 1973). All spores tested for viability were from specimens collected in 1982. After breaking the walls mechanically the spores were stained with acetocarmine. The relationship between stained and nonstained spores with broken walls was independent of spore shape. Results are shown in Table 6. In all cases the percentage of stained spores was above 70%. The majority of spores exhibited cellular content when they were subjected to the Wodehouse method (Fig. 3). Thus, all specimens had viable (living) spores, regardless of spore shape.

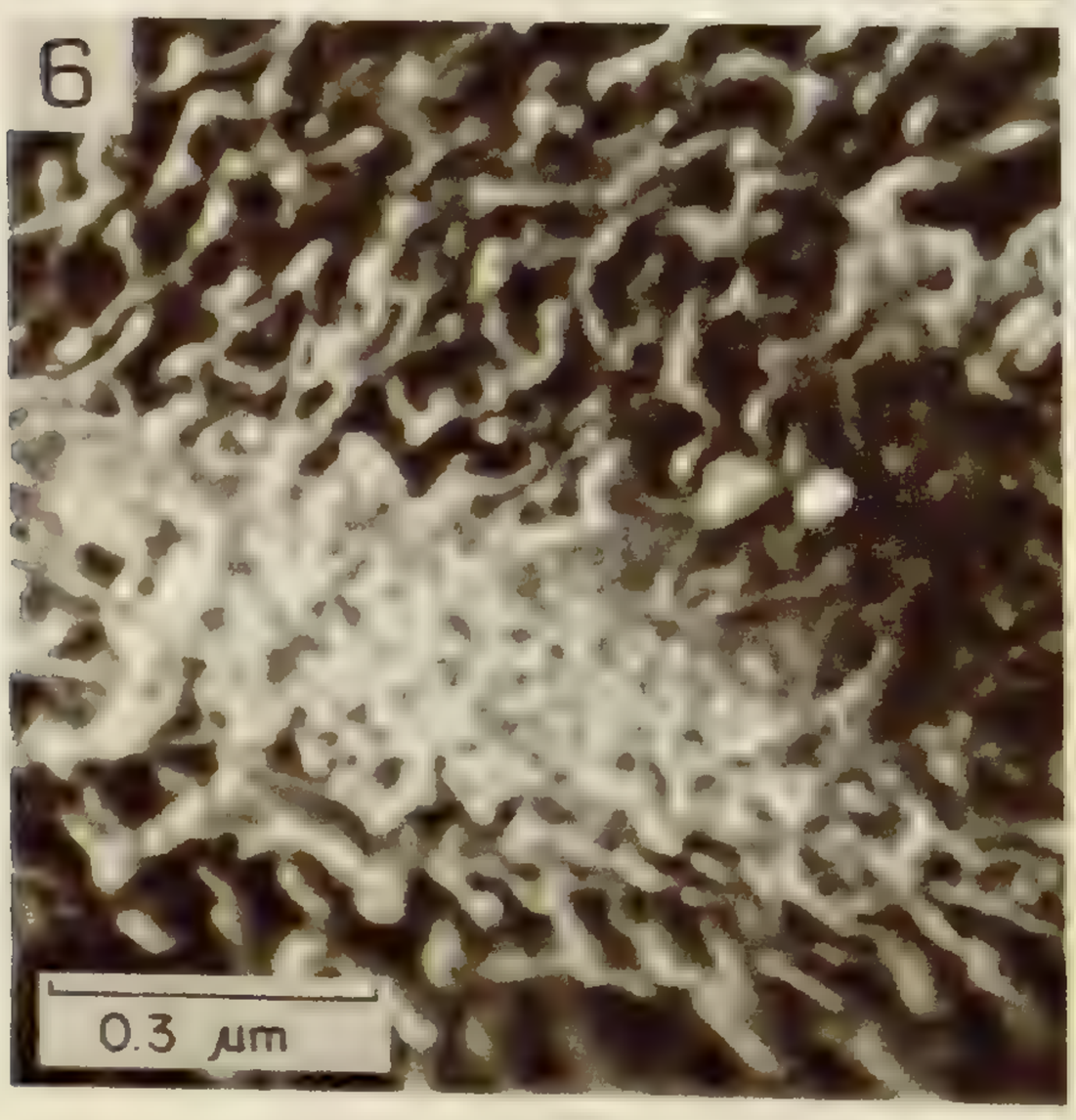
TABLE 6. Viability of Freshly Collected Spores of Five Specimens of *Trichipteris corcovadensis*, Collected in 1982. Spores Were Stained with Acetocarmine After Having Their Walls Broken Mechanically.

Specimen	Viability (%)
A	88
B	79
C	86
D	77
E	76









FIGS. 3–7. Spores of *Trichipteris*. FIG. 3. Specimen C treated by the Wodehouse method, showing the cellular content. FIG. 4. Surface view of the spore of specimen E, scanning electron photomicro-

TABLE 7. Total Lipid and Soluble Protein in Spores of Five Specimens of *Trichipteris corcovadensis* Collected in Different Years.

Specimen—year	mg·100 mg^{-1} spores	
	Lipid*	Protein*
A—1982	44.2	1.14
B—1981	47.5	1.01
B—1982	48.8	0.93
C—1982	50.1	1.11
C—1983	45.0	—
D—1982	—	0.98
E—1982	—	1.02

* $F_{5\%}$ not significant for lipids and protein.

The content of total lipids and soluble protein was determined in spores collected in different years. About 47% of the weight of the spores is lipid and only 1% is protein (Table 7). No differences could be seen in lipid and protein content in the different specimens studied. Gemmrich (1977, 1982) showed similar values for lipids (over 59% by weight) with spores of *Anemia phyllitidis*. According to Cran (1979), 20% by weight of spores of *Dryopteris pseudomas* is lipid.

Measurements of the equatorial diameter and the trilete length (see Fig. 2A) of spores after acetolysis are shown in Table 8. Spores from specimen A showed the largest diameter, but this diameter was not statistically different from that of spores from specimen B. In all cases the length of the trilete is about 1.6 times smaller than the equatorial diameter independent of the spore.

TABLE 8. Equatorial Diameter and Trilete Length of Five Specimens of *Trichipteris corcovadensis* Collected in Different years.

Specimen—year	Equatorial diameter $\bar{x}$ µm		Trilete length $\bar{x}$ µm	
	Shape I	Shape II	Shape I	Shape II
A—1982	73.1^{a}	—	46.0^{a}	—
B—1980	57.8^{d}	57.0^{d}	35.9^{c}	34.8^{c}
B—1981	71.5^{a}	57.8^{d}	46.3^{a}	45.0^{ab}
B—1982	64.2^{bc}	58.3^{d}	41.6^{b}	37.6^{c}
C—1982	65.5^{b}	57.4^{d}	40.5^{bc}	37.4^{c}
D—1982	68.5^{ab}	61.1^{cd}	43.9^{ab}	37.3^{c}
E—1982	68.5^{ab}	62.3^{c}	44.0^{ab}	37.7^{c}
$LSD_{5\%}$ (Tukey)	3.2		3.6	
Coefficient of variation	5.4%		9.8%	

graph. FIG. 5. Surface view of specimen D, scanning electron photomicrograph. FIG. 6. Surface view of specimen B showing the perine, scanning electron photomicrograph. FIG. 7. Perine of specimen B after treatment with sodium carbonate, as shown in the optical microscope.

TABLE 9. Main Aspects of the Surface of Spores of Five Specimens of *Trichipteris corcovadensis*.

Specimen	Main sculpture	Pits near trilete scar
A	granulate ?	evident
B	granulate ? or pitted, intercalated with small granules	evident
C	psilate, clearly pitted	smaller near the trilete scar
D	psilate, delicately pitted	absent
E	psilate, with small and few pits	evident

The surface of the spores was observed through the optical and the scanning electron microscopes. The sculpture is variable and variations occur not only among the five specimens but also in the same sample. An idea of this variation is given in Table 9, but even so it was possible to define the main sculpture pattern of the spores from each specimen. For example, spores from specimen A show a granulate sculptine while specimen D exhibits a psilate pattern. Specimen E shows the trilate scar demarcated by pits (Fig. 4), which are not observed in spores from specimen D (Fig. 5).

According to Gastony (1974), the perine is lacking in many species of *Trichipteris*. In the present case, perine was observed under both the scanning (Fig. 6) and optical microscopes (Fig. 7). Data presented in Table 10 were obtained with the optical microscope (spores treated with sodium carbonate before acetolysis). It can be seen in Table 10 that 94% of spores of specimen A showed immature granulate perine. Perine at different stages of development was present in spores independent of their shape.

According to Gastony & Tryon (1976) perinous sculptine is attained quite late in spore maturation. They state that "when dried, very immature spores will collapse as the result of incomplete development of mature wall structure and such spores will appear pinched in microscopy". Maturation is a continuum, and even though the mature wall structure will preclude the pinched appearance upon drying, the late development in the final perine is not attained yet. Spores

TABLE 10. Frequency of Spores with Perine of *Trichipteris corcovadensis* Collected in Different Years.

Specimen—year	Perine (%)
A—1982	94[a]
B—1980	79[c]
B—1981	86[b]
B—1982	81[c]
C—1982	74[d]
D—1982	78[cd]
E—1982	67[e]
$LSD_{5\%}$ (Tukey)	4.1

not quite fully mature will present different stages of perine deposition (Gastony & Tryon, 1976). They rely on their experience to judge immature, partially mature, or fully mature perines.

Taking into account the work of Gastony (1979), all spores of specimens B, C, D, E and most of spores from A are immature. None of the spores of specimen A present the perine as hair-like processes, considered by Gastony (1979) as a feature of mature perine, which identifies a mature spore. However, some of the immature spores of specimen A germinate, showing that even though they are not fully mature morphologically, they have reached maturity from a physiological point of view. The majority of spores were viable (i.e., stainable), but only the almost mature ones (morphologically) of specimen A actually germinated.

L.M.E. thanks Fundação de Amparo à Pesquisa do Estado de São Paulo for grant 80/462:2. The authors thank Dr. L. Sodek for correcting the English and Mrs. Luzia M. Kiyono for the typing.

Literature Cited

Barrington, D. S. 1978. A revision of the genus *Trichipteris*. Contr. Gray Herb. 208:1–93.

Bewley, J. D. and M. Black. 1978. *Physiology and biochemistry of seeds in relation to germination.* Berlin: Springer-Verlag.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 72:248–254.

Cran, D. C. 1979. The ultrastructure of fern gametophyte cells. In *The experimental biology of ferns*, ed. A. F. Dyer. London: Academic Press.

Cruz, M. A. V. and T. S. Melhem. 1984. Estudos polínicos em Sapindaceae. Revista Brasil. Bot. 7: 5–25.

Dyer, A. F. 1979. The culture of gametophytes for experimental investigation. In *The experimental biology of ferns*, ed. A. F. Dyer. London: Academic Press.

Erdtman, G. 1969. *Handbook of palynology (Morphology-Taxonomy-Ecology). An introduction to the study of pollen grains and spores.* Copenhagen: Munksgaard.

——— and P. Sorsa. 1971. *Pollen and spore morphology/plant taxonomy*. Stockholm: Almqvist & Wiksell.

Gastony, G. J. 1974. Spore morphology in the Cyatheaceae. I. The perine and sporangial capacity: general considerations. Amer. J. Bot. 61:672–680.

———. 1979. Spore morphology in the Cyatheaceae. III. The genus *Trichipteris*. Amer. J. Bot. 66: 1238–1260.

——— and R. M. Tryon. 1976. Spore morphology in the Cyatheaceae. II. The genera *Lophosoria, Metaxya, Sphaeropteris, Alsophila* and *Nephelea*. Amer. J. Bot. 63:738–758.

Gemmrich, A. R. 1977. Mobilization of reserve lipids in germinating spores of fern *Anemia phyllitidis* L. Pl. Sci. Lett. 9:301–307.

———. 1982. Effect of red light and gibberellic acid on lipid metabolism in germinating spores of fern *Anemia phyllitidis*. Physiol. Pl. (Copenhagen) 54:58–62.

Kremp, O. W. and T. Kawasaki. 1969. *The spores of the pteridophytes*. Tokyo: Hirokawa Publishing Company, Inc.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.

Marcondes-Ferreira, W. M. and G. M. Felippe. 1984. Effects of light and temperature on the germination of spores of *Cyathea delgadii*. Revista Brasil. Bot. 7:53–56.

Melhem, T. S. and M. E. R. Matos. 1972. Variabilidade de forma nos grãos de pólen de *Eriope crassipes* Benth—Labiatae. Hoehnea 2:1–10.

Miller, J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–440.

MORBELLI, M. A. 1974. Analisis palinologico en hibridos interespecíficos del genero *Blechnum* L. Bol. Soc. Argent. Bot. 15:446–466.

RIZZINI, C. T. 1973. Dormancy in seeds of *Annona crassiflora*. J. Exp. Bot. 24:117–123.

SALGADO-LABOURIAU, M. L., P. E. VANZOLINI, and T. S. MELHEM. 1965. Variation of polar axes and equatorial diameters in pollen grains of two species of *Cassia*. Grana Palynol. 6:166–176.

SNEDECOR, G. N. 1962. *Statistical methods*. Ames: The Iowa State University Press.

TRYON, R. M., JR. 1970. The classification of the Cyatheaceae. Contr. Gray Herb. 200:3–53.

WODEHOUSE, R. P. 1935. *Pollen grains. Their structure, identification and significance in science and medicine*. New York: McGraw-Hill Book Company.

SHORTER NOTE

New Locations for *Isoëtes tegetiformans* in Georgia.—Since its discovery in one pool on Heggies Rock, Columbia County, Georgia, in 1976, and its description in 1978 (Rury, Amer. Fern J. 68:99–108), fourteen more pools of *I. tegetiformans* Rury have been found in Columbia, Greene, Hancock, and Putnam Counties, Georgia (Fig. 1). James R. Allison of Lawrenceville, Georgia, discovered these populations at six new sites during 1978 and 1979, while exploring some 150 flatrock exposures in four states.

In May 1978, Dr. James G. Bruce and I visited the first of these new localities (Greene Co.) with Mr. Allison and confirmed the presence of *I. tegetiformans* in five different pools. In a December 1979 letter to me, Mr. Allison reported

FIG. 1. *Isoëtes tegetiformans* in Georgia. Distribution map modified from J. R. Allison's 1979 letter. Encircled star and associated map coordinates designate Heggies Rock, the type locality. Stars denote new localities; numbers in parentheses indicate the number of populations (pools) found at a given locality. Solid circles denote granite outcrops searched but lacking populations.

FIGS. 2 and 3. *Isoëtes tegetiformans*. 2, Type population at Heggies Rock. Southern aspect of this solitary pool shows the low rim or "spillway" that minimizes soil and water retention. Only the north flank of the pool is colonized by mats of *Polytrichum*, *Selaginella tortipila* A. Braun and *Andropogon scoparius* L. (21 Jan 1978). 3, The most densely populated pool at the Greensboro flatrock locality. Eastern aspect of pool shows characteristic spillway and granitic rubble. Note the lack of encroaching mosses and grasses along the rim. (Jim Allison and Jim Bruce, left to right, 13 May 1978.)

having found another nine pools of *I. tegetiformans* from five new flatrock sites in Columbia, Hancock, and Putnam Counties, and sent specimens that confirmed his discoveries. Since then, I have been unable to locate Mr. Allison, who to my knowledge has not published these data. Therefore, this note was prepared for the benefit of others interested in the distribution of this unique, mat-forming *Isoëtes*.

Isoëtes tegetiformans is restricted to shallow, summer-dry pools on porphyritic granitic flatrocks. Moreover, populations may occur sympatrically with the more widespread *I. piedmontana* (Pfeiffer) Reed, which typically is confined to deeper soils in seepage areas and in open woodland communities along the margins of these same flatrocks. Photographs of the type population at Heggies Rock (Fig. 2) and a pool of *I. tegetiformans* at the Greensboro flatrock (Fig. 3) are presented to aid its recognition in the field.

The following locations and the distribution map (Fig. 1) for all known populations of *I. tegetiformans* are based primarily on Mr. Allison's 1979 letter.

GEORGIA: **Columbia Co.**: two pools at quarry site near Little Kiokee Creek, S of Ga. Rte. 232 and ca. 2.4 km S of Heggies Rock, Appling, 106 m, *Allison et al. 949* (GA); one pool on flatrock ca. 1.07 km W of Little Kiokee Creek site, elev.?; Two pools on flatrock ca. 2.14 km W of the Little Kiokee Creek site, elev.?. **Greene Co.**: five pools at "Greensboro flatrock" ca. 16 km SSE of Greensboro and SE of the junction of State Road 925 and County Road 56 (33°28′30″N, 83°08′W), 185 m. *Allison et al. 2* (GA); *Allison 647* (GA); *Jones et al. 22868* (GA). **Hancock Co.**: two pools on granite flatrock on the Boone property, at end of dirt road off Ga. Rte. 15 ca. 7.2 km SE of Sparta, elev. ?, *Allison 1173* (NCU); *Allison & Murphy 1310* (GA). **Putnam Co.**: two pools on large granitic dome on Lake Wallace, ca. 16 km SW of the Greensboro flatrock site and ca. 1.28 km W of the junction of Greene, Hancock, and Putnam Counties, 139 m *Allison & Murphy 1132* (GA).

PHILLIP M. RURY, Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138.

REVIEW

"*Guide de fougères et plantes alliées*," by Rémy Prelli. 1985. 199 pp. Paris: Lechevalier. ISBN 2-7205-0516-1. (hardbound).

A general discussion of the biology and classification of ferns and fern allies, followed by an illustrated floristic account of French pteridophytes.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

AMERICAN FERN JOURNAL

Volume 75

Number 4

October–December 1985

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Relationships between Ecological Pattern and Branching in the Tree Fern *Lophosoria quadripinnata* in Veracruz, Mexico — *Paul Alan Cox* and *P. B. Tomlinson* — **105**

Ontogeny of the Commissure of *Equisetum* — *Richard L. Hauke* — **111**

Wild Gametophytes of *Equisetum sylvaticum* — *Jeffrey G. Duckett* — **120**

Osmunda *cinnamomea* forma *frondosa* at Mountain Lake, Virginia — *Charles R. Werth, Melanie L. Haskins,* and *Akke Hulburt* — **128**

Shorter Notes

Gametophytes of *Equisetum giganteum* — *Richard L. Hauke* — **132**

***Marsilea macropoda* New to Louisiana** — *Garrie P. Landry* and *Samuel W. Holder* — **133**

Review — **119**

E. A. C. L. E. Schelpe (1924–1985) — **133**

Announcement: AIBS Meeting—Call for Papers — **134**

Referees, 1985 — **134**

Index to Volume 75 — **134**

Information for Authors — **Cover 3**

MISSOURI BOTANICAL

MAR 11 1986

The American Fern Society

Council for 1985

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268. *President*

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH . . . . . Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY . . . . . Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER . . . . . Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER . . . . . U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER . . . . Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 75(4):105–110 (1985)

Relationships between Ecological Pattern and Branching in the Tree Fern *Lophosoria quadripinnata* in Veracruz, Mexico

PAUL ALAN COX
Department of Botany and Range Science, Brigham Young University, Provo, UT 84602
P. B. TOMLINSON
Harvard Forest, Harvard University, Petersham, MA 01366

Pteridophytes often play important roles in determining the nature of plant communities (Watt, 1947). This role is particularly pronounced in tropical cloud forests where the diversity and variety of growth forms in ferns often is very great. In this paper we describe an investigation between the relationship of branching and patterns of shoot distribution in a tree fern.

We chose to study the distribution pattern of shoots of *Lophosoria quadripinnata* (J. Gmelin) C. Chr. (Lophosoriaceae), a small tree fern that occurs throughout the Neotropics (Tryon & Tryon, 1982), and attempted to relate the observed pattern of shoot distribution to the morphology and branching pattern of the species. The shoot morphology of this species has been described superficially by earlier authors. Bower (1926) illustrated a short segment of the axis and showed that the aboveground stems arise from a rhizome. Lucansky (1982) limited his comparison of the anatomy of *Lophosoria* and *Metaxya* to the erect stems. We therefore sought to understand the very prominent role *Lophosoria quadripinnata* plays in cloud forest vegetation by futher investigating its morphology and ecology.

METHODS

Previous workers (Tryon et al., 1973) had made extensive maps of pteridophyte vegetation occurring in the cloud forest at Pas de Enriques in the state of Veracruz, Mexico, an area that occurs on the steep slopes of the escarpment of the Sierra Madre Oriental at an elevation of about 1350 meters. The shoot distribution of *Lophosoria quadripinnata* at Pas de Enriques was initially studied by mathematical analysis of these maps; subsequently, field investigations of *Lophosoria quadripinnata* were made and material for morphological analysis was collected.

The original maps compiled by Tryon et al. (1973) were sampled with a paper quadrat scaled so as to yield data comparable with a transect of 32 contiguous meter-square quadrats. In each quadrat, the presence or absence of *Lophosoria quadripinnata* was recorded. Ten transects of this type were analyzed for spatial heterogeneity of shoots using a one-dimensional pattern analysis, a variation of the two-dimensional technique described by Greig-Smith (1957). First, a variance was calculated for the original transect by using the formula

$$\sigma = \frac{\sum_{i=1}^{n} (x - \bar{x})}{n - 1}$$

where x is the value for *Lophosoria quadripinnata* (1 for present, 0 for absent) in the ith quadrat, $\bar{x}$ is the mean value for all quadrats, and n is the number of quadrats. Since this variance is calculated for samples taken using a meter-square quadrat, the value of the variance is plotted against a 1 meter block size. The 32 quadrat transect is then reanalyzed by considering it to represent 16 contiguous 2 meter by 1 meter quadrats. Presence and absence are then recorded for each of these new rectangular quadrats, and a new variance calculated. The value of this new variance is then plotted on the same graph against a 2 meter block size. The original 32 quadrat transact is then considered to consist of 8 contiguous 4 meter by 1 meter quadrats. Presence and absence are calculated for each of these 4 meter by 1 meter quadrats, and the new variance is then plotted against an 8 meter block size. This procedure is then repeated for a 16 meter block size. The resulting graphs (Fig. 1) yield significant information concerning shoot distribution, for a peak in the graph indicates significant spatial heterogeneity or pattern at that particular block size (Greig-Smith, 1957).

Subsequent to the analysis of the maps, field studies of a population of *Lophosoria quadripinnata* were conducted at Pas de Enriques. Erect shoots were not found to be uniformly distributed in the vegetation, but instead were grouped into distinct clumps or patches, each clump appearing in the vegetation as a dense mass of leaves. The maximum dimensions parallel and perpendicular to the fall line for 10 such patches were recorded. In addition, a large patch was completely excavated and a map of the rhizome system indicating all erect and horizontal shoots was drawn. The unearthed rhizome system then was sectioned, color-coded for reassembly, washed, photographed, preserved in FAA, and analyzed in the laboratory.

Approximately two weeks later the rhizomes were sequentially sectioned and filmed in frame-by-frame analysis. Each sawn surface was photographed by means of a cine camera after it was planed on a radial-arm saw in a modification of the basic cinematographic technique described by Tomlinson (1970) and Zimmermann (1976). The resulting motion picture was analyzed for details of morphology, including branching of axes, and anatomy, including stelar pattern characterizing leaf and branch insertion.

Results

Eight of the ten transects indicated significant ecological pattern as shown in Figure 1 which consists of graphs of transect variances plotted against block sizes. The mean block-size at which significant pattern occurs was found to be 4.25 meters. Of the remaining two transects, one did not exhibit pattern at any block size for *Lophosoria* and the other did not include any *Lophosoria* trunks.

The rhizomatous growth of *Lophosoria* appears to be one of the major factors generating the observed ecological pattern. Although large patches measuring

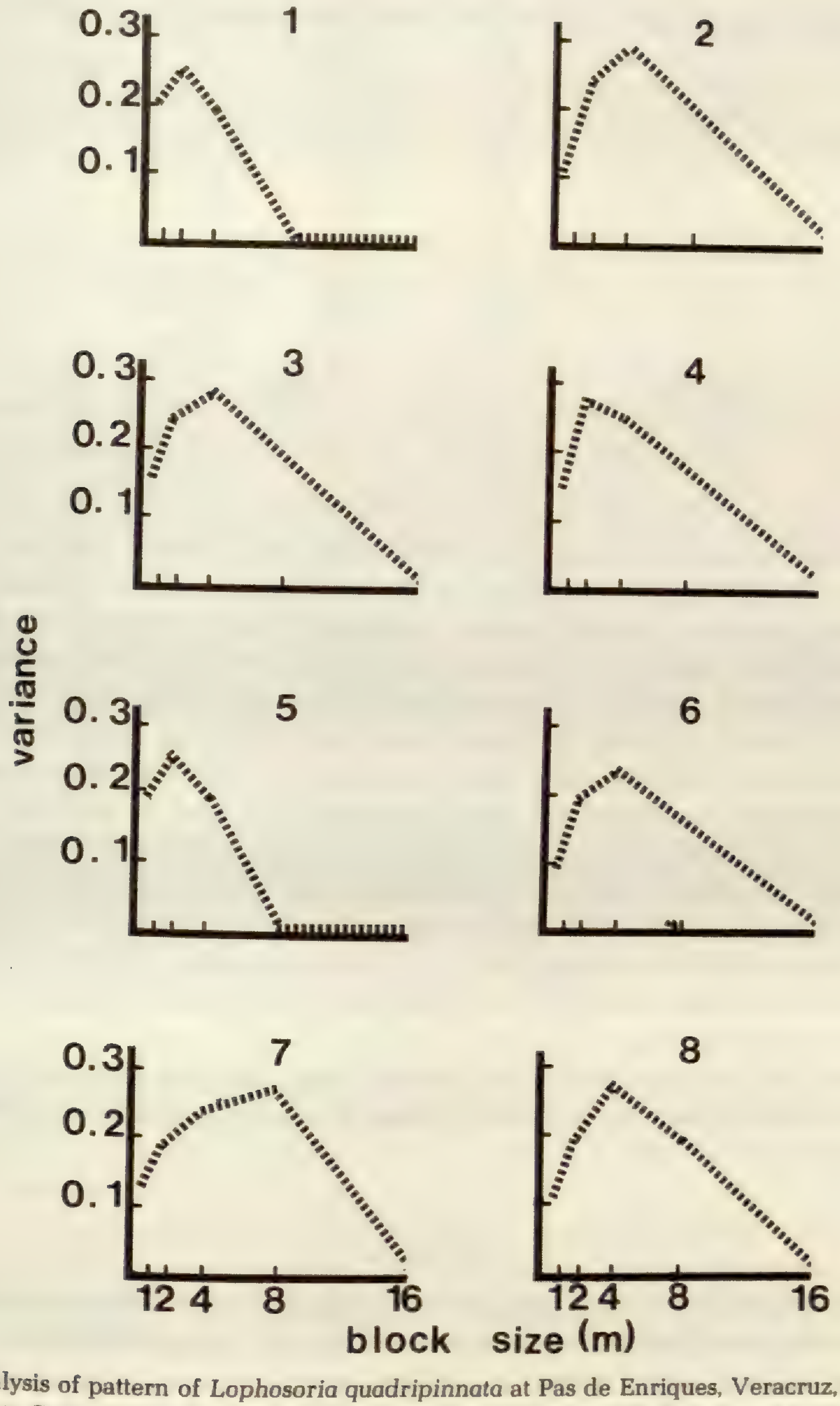


FIG. 1. Analysis of pattern of *Lophosoria quadripinnata* at Pas de Enriques, Veracruz, Mexico, for eight transects. For each graph block size is plotted along the x axis while variance is plotted against the y axis.

up to 19 meters in diameter and occupying over 280 square meters were recorded, the mean diameter of a *Lophosoria* patch as measured in the field (Table 1) is 5.97 meters, a value that would be detected in the pattern analysis as a peak at a block size of either 4 or 8 meters.

Our morphological analysis confirms that *L. quadripinnata* has a dimorphic shoot system consisting of underground plagiotropic axes (rhizomes) that bear

TABLE 1. Diameters of *Lophosoria quadripinnata* Clones on a Vertical Projection. Mean = 5.97 m; s.d. = 5.18 m.

Clone #	Diameter
1	2.75
2	4.90
3	2.97
4	1.47
5	5.20
6	2.70
7	4.87
8	12.56
9	4.40
10	17.89

only scale leaves and aboveground orthotropic axes (trunks) that bear foliage leaves (Fig. 2). The scale leaves have no indication of a rachis or a blade. Each rhizome originates as a branch from an existing rhizome, grows horizontally for an extended period, and then becomes erect as a leafy axis that emerges as the visible trunk of the fern. The sympodium is proliferated by branch rhizomes that arise at intervals along the parent rhizome. Branches develop from buds located abaxial to the scale leaves on the rhizome, but the frequency of bud development does not follow any regular pattern. However, branches occur most often at the distal end of rhizomes in the region where the axis becomes orthotropic. The erect aerial stem does not itself branch. Branch and leaf insertion can be distinguished readily in transverse section. The leaf trace of a scale leaf makes a characteristic U-shaped leaf gap in the vascular cylinder of the stem, while the branch trace forms no gap, the branch being completely solenostelic from its level of initiation.

Phyllotaxis in the rhizome is spiral with a mean angular divergence of 135.4 degrees (n = 69, s.d. = 28.5) but may change in axes that exhibit transition from plagiotropic to orthotropic growth.

DISCUSSION

The analysis of pattern is a valuable technique for detecting non-randomness in the spatial arrangement of vegetation. The determination of the scale or block size at which significant pattern occurs allows hypotheses to be made concerning factors that control that pattern. In our study of *Lophosoria quadripinnata*, we found significant pattern to exist at a mean block size of 4.25 meters. Since no features of the terrain or soils of the site were known to exhibit a pattern at that level and since none of the other pteridophyte species recorded by Tryon et al. (1973) showed pattern at that level, we hypothesized that exogenous factors were not responsible for the pattern detected in *L. quadripinnata*. Our hypothesis instead was that an endogenous factor, specifically a rhizomatous growth habit, was responsible for the detected pattern. However recent workers (Lucansky, 1974, 1982; Lucansky & White, 1974) had not studied decumbent axes in *L. quad-*

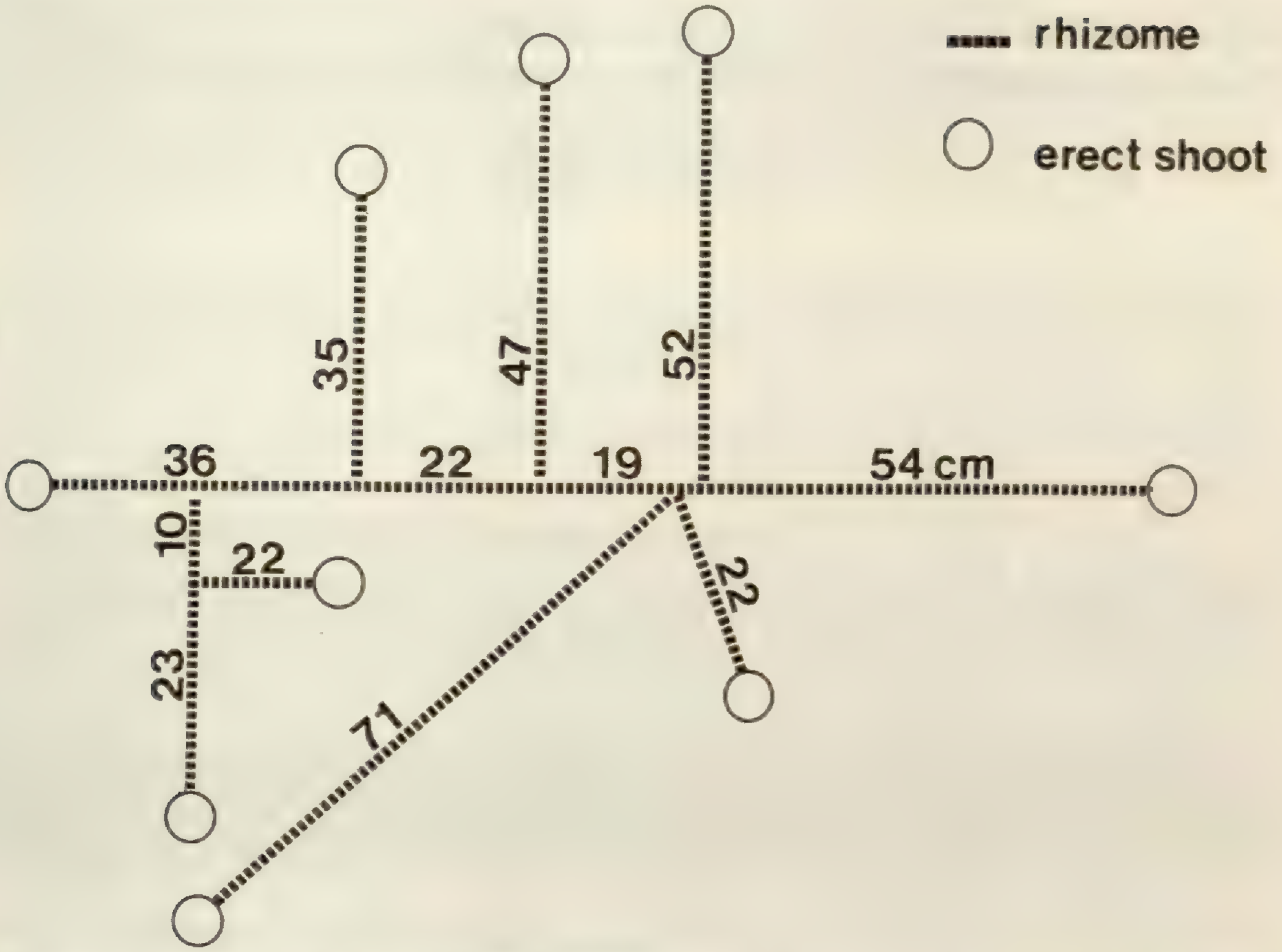


FIG. 2. Diagrammatic map of rhizome system excavated of *Lophosoria quadripinnata*. Distances given in centimeters. Angles of branching shown are stylized and do not represent actual divergence of branches.

ripinnata although Bower (1926, p. 286; 1912, p. 293) was aware of their existence. Therefore field studies were necessary to test our hypothesis that rhizomatous growth is a prominent feature of *L. quadripinnata* growth. Our studies confirmed the existence of a rhizomatous growth pattern in *L. quadripinnata* while the measurement of patches revealed patch size to be consistent with the scale of pattern detected in our pattern analysis. It therefore appears likely that rhizomatous growth is the major factor determining ecological pattern of *Lophosoria quadripinnata* at Pas de Enriques. However it is also possible that patch size and pattern reflect some unknown feature of sexual reproduction (Conant & Cooper-Driver, 1980); this alternative cannot be tested with our data.

Rhizomes are known from other tree fern genera such as *Cyathea sternbergii* Pohl (Brade, 1971) and *Alsophila marriana* (Hook.) Tryon (Hallé, 1966) while *Cyathea parvula* (Jenm.) Dunim produces negatively geotropic branches that root when they reach the soil (Tryon & Tryon, 1982). It is not known how these features affect their ecological pattern.

Pattern analysis has not been used previously in ecological studies of pteridophytes with the exception of Anderson's (1961) work on *Pteridium aquilinum* in North Wales. That study is notable in that it examines the overall pattern of clonal development of fern rhizomes (cf., Bell & Tomlinson, 1980). Certainly pattern analysis has proven useful in our study of *Lophosoria quadripinnata* and

we commend its use to ecologists and morphologists as a means of generating hypotheses concerning the relationship of ecology and morphology in ferns.

Acknowledgments

We thank R. Tryon and A. Tryon for useful discussions and two anonymous reviewers for useful criticisms of our manuscript. M. Moore assisted in data collection. During this study Cox was funded by a Danforth Fellowship and a National Science Foundation Graduate Fellowship. Field work in Mexico was supported by the Atkins Garden Fund, Harvard University.

Literature Cited

Anderson, D. 1961. The structure of some upland plant communities in Caernarvonshire. I—The pattern shown by *Pteridium aquilinum*. J. Ecol. 49:369–377.

Bell, A. D. and P. B. Tomlinson. 1980. Adaptive architecture in rhizomatous plants. J. Linn. Soc., Bot. 80:125–160.

Bower, F. O. 1912. Studies in the phylogeny of the Filicales. IV. *Lophosoria* and its relation to the Cyatheoidea and other ferns. Ann. Bot. 26:269–324.

———. 1926. *The ferns*. Vol. II. London: Cambridge University Press.

Brade, A. C. 1971. *Cyathea sampaioana* Brade et Ras. Somente uma "forma" de *Cyathea sternbergii* Pohl. Bradea 10:73–76.

Conant, D. S. and G. Cooper-Driver. 1980. Autogamous allohomoploidy in *Alsophila* and *Nephelea* (Cyatheaceae): a new hypothesis for speciation in homoploid homosporous ferns. Amer. J. Bot. 67:1269–1288.

Greig-Smith, P. 1957. *Quantitative plant ecology*. London: Butterworth.

Hallé, F. 1966. Étude de la ramification du tronc chez quelques fougères arborescentes. Adansonia 6:405–424.

Lucansky, T. W. 1974. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. I. *Metaxya* and *Lophosoria*. Amer. J. Bot. 61:472–480.

———. 1982. Anatomical studies on the neotropical Cyatheaceae. II. *Metaxya* and *Lophosoria*. Amer. Fern J. 72:19–29.

——— and R. A. White. 1974. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. III. Nodal and petiole patterns: summary and conclusions. Amer. J. Bot. 61:818–828.

Tomlinson, P. B. 1970. Monocotyledons: towards an understanding of their morphology and anatomy. Pp. 207-292 in *Advances in botanical research*, Vol. 3, ed. R. D. Preston. London: Academic Press.

Tryon, R., B. Voeller, A. Tryon, and R. Riba. 1973. Fern biology in Mexico. BioScience 23:28–32.

——— and A. Tryon. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.

Watt, A. 1947. Contributions to the ecology of bracken. IV. The structure of the community. New Phytol. 46:97–121.

Zimmermann, M. H. 1976. The study of vascular patterns in higher plants. Pp. 221–235 in *Transport and transfer processes in plants*, eds. I. E. Wardlaw and J. B. Passioura. New York: Academic Press.

American Fern Journal 75(4):111–119 (1985)

Ontogeny of the Commissure of *Equisetum*

RICHARD L. HAUKE
Department of Botany, University of Rhode Island, Kingston, RI 02881

The genus *Equisetum* is characterized by having whorled leaves fused into a nodal sheath. This sheath consists of a variable number of segments, or leaves, three in *E. scirpoides* to 50 or more in *E. giganteum*. Each segment has a single vascular bundle, continuous with a vascular bundle of the internode below it, and is terminated by a tooth, or free leaf tip. Between adjacent segments there is an anatomically distinct commissure.

This commissure was first described by Duval-Jouve (1864) as consisting of transversely elongated cells at the bottom of the commissure bordered by oblique to irregular cells, as opposed to the vertically elongate cells of the sheath epidermis. Milde's (1867) description was a little more complete. There is a row of transversely elongated cells lying one above another, which have strongly thickened walls. Their ends bend a little downward, giving them a half-moon shape. Neighboring cells overlap them, and this overlapping may be so strong as to completely cover the cells of the commissure. Müller (1888) provided an extended discussion of the commissural appearance. He named the transversely oriented row of cells "anchor cells" (*ankerzellen*) because they anchor the adjacent sheath segments. With the adjacent obliquely oriented cells they form parabolic curves he compared to "chain lines" (*kettenlinien*), which are formed by suspending a chain between two upright supports. With descriptions, mathematical formulae, and polarized light analyses of cell walls, he supported his theory that the characteristic form of the commissure is the result of rapid elongation of the sheath teeth stretching the still meristematic cells of the commissural region.

He described the appearance of the commissure in cross-section and the variation between species. The anchor cells have swollen ends and thick walls. They are underlain by small, thick-walled cells of the inner epidermis. In some species the commissure is shallow, in others deep, and in some the sides of the commissure overhang the furrow and partly to completely occlude it.

Mature anchor cells bend at the ends transversely outward. The wall is thick, but thinnest at the bend. According to Müller this makes the commissure stretchable. The sheath functions to protect the growing point and developing sheaths, and its stretchability allows them to elongate through it, and provides flexible protection and mechanical strength for the intercalary meristems at the base of the internode above the sheath.

Müller (1888) described the ontogeny of the anchor cells in *E. hyemale*. At the commissure the young cells are 2–4 layers thick, descended from one or two meristem cells. Over them lies a single, large, somewhat 6-sided cell, which belongs to the outer epidermis. The inner wall is about twice as long as the outer wall. The lateral neighbors of the anchor cell are meristematic and divide, so

that the sides overgrow the anchor cell, until the commissural groove is a mere slit (Fig. 15). The outer wall of the anchor cell becomes as big as the inner wall. Just under the anchor cell a cell is cut off, either from the underlying cells or from the anchor cell itself, which may divide once or more, and which forms a pillow for the anchor cell. Finally the ends of the anchor cell grow out and gradually bend around, forming the anchor shape. Then the walls become thick, and thus is established the deceptive appearance of an anchor cell lying deep in the inside of a closed tissue complex. The nucleus of the young anchor cell is larger than those of the adjoining cells and is elliptical. As the cell ends grow out, the nucleus remains right in the middle of the lengthwise axis, coming to lie against the outside wall and becoming spindle-shaped. According to Müller the rhizome sheaths have no such distinctive commissure.

Schellenberg (1895) disagreed with Müller, and attributed the characteristic appearance of the commissure to unequal growth at the base rather than teeth enlargement. He pointed out that the teeth have elongated before the commissure forms. The lower part of the sheath segment is more actively growing than is the lower part of the anchor cell row. Upper cells of the anchor cell row accommodate this unequal growth by stretching into parabolic curves. Sadebeck (1902) described the establishment of the anchor cell row by division from the neighbor cells. As the sheath grows, it forms new anchor cells, but only from below. He cited Müller's explanation of tooth growth causing stretching of cells.

More recent authors have not described the commissure. DeBlock (1923) speaks of the sheath segments being "concrescent," Eames (1936) describes the leaves as "fused laterally," Smith (1955) says they are "laterally united," Bierhorst (1971) calls them "connate," and having "a single row of hook-shaped cells that seem to clamp the adjacent leaves together," Foster and Gifford (1974) say they are "united," and Bold et al. (1980) "fused."

I (1979) observed variations in appearance of the commissures of species of subgenus *Equisetum*, and have since studied the anatomy and ontogeny of the commissure of *Equisetum*. The distinctive row of cells in the middle of the commissure will be called the C-cells here, short for commissure, rather than anchor cells, which implies a function which may be misleading.

Materials and Methods

Buds of *Equisetum arvense*, *Equisetum diffusum*, *E. hyemale*, and *E. telmateia* were killed and fixed in FAA, transferred to 70% EtOH, dehydrated in a tertiary butyl alcohol series, embedded in Paraplast, (mp 56–57°C) and sectioned on a rotary microtome at 10 μm (longitudinal sections), or 20 μm (transverse sections). These were stained with aqueous ferric chloride and tannic acid (the reverse of the usual sequence of Foster, 1934), counterstained with .01% toluidine blue in 95% EtOH, and mounted in Diaphane. Some fixed buds were cleared with 10% KOH followed by chloral hydrate, stained with tannic acid and ferric chloride, teased apart in Diaphane and mounted on slides. The photos were taken on Kodak Plus-X or Technical Pan film with a Nikon Microflex UFX camera mounted on a Zeiss microscope. Sources of plant material are as follows:

Equisetum arvense.—Moat in front of main library, University of Rhode Island campus, Kingston, 31 Aug 1974 (Voucher: Hauke 514, KIRI).

E. diffusum.—Pot in University of Rhode Island, Botany Department greenhouse, Kingston, 10 Jan 1985. Plant originally collected near Simla in Himchal Pradesh state, India (Voucher: Hauke s.n., KIRI).

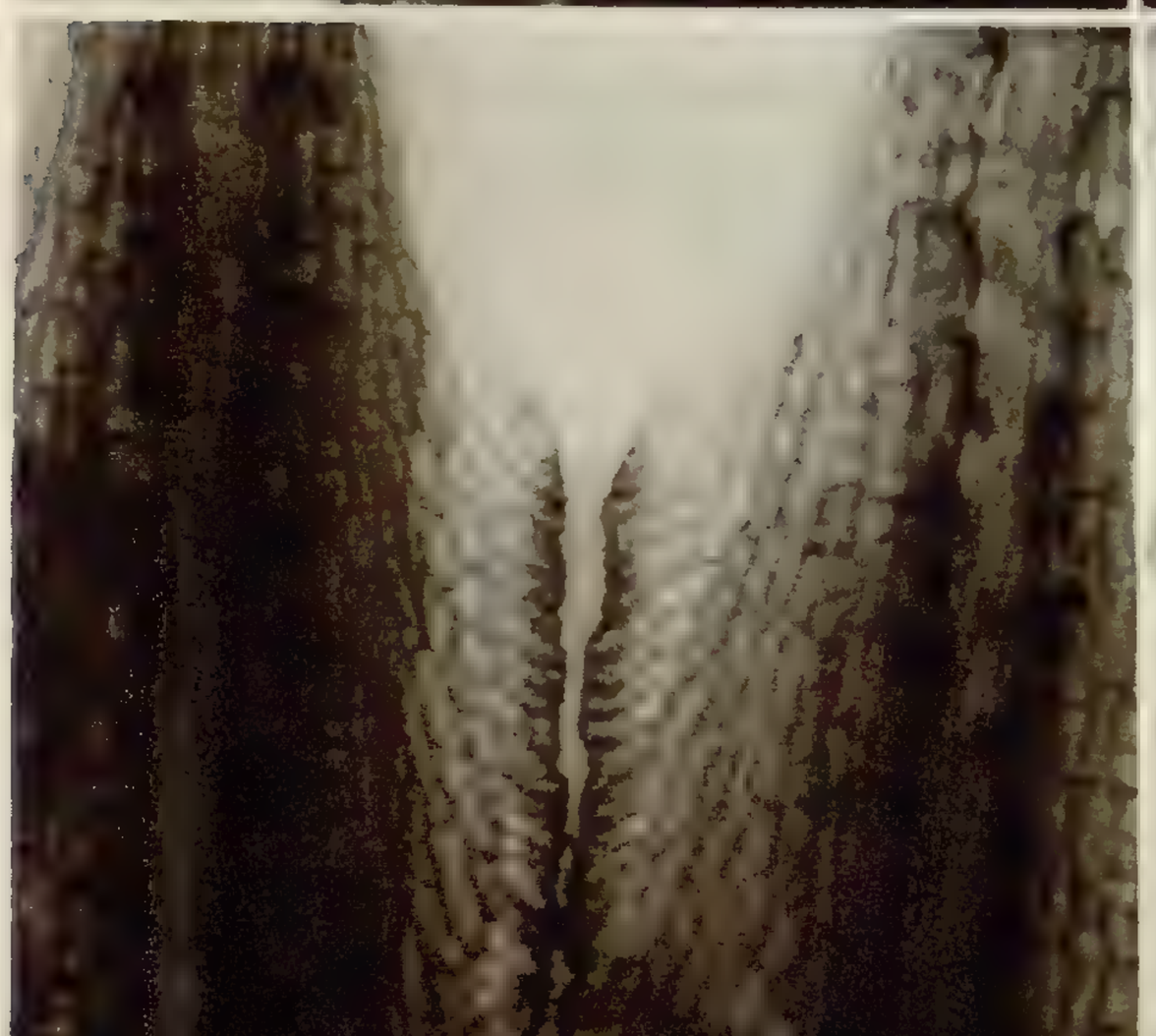
E. hyemale.—Northeast corner of Galilee Escape Rd. and Great Island Rd., Narragansett, Rhode Island, 31 Dec 1984 (Voucher: Hauke 515, KIRI).

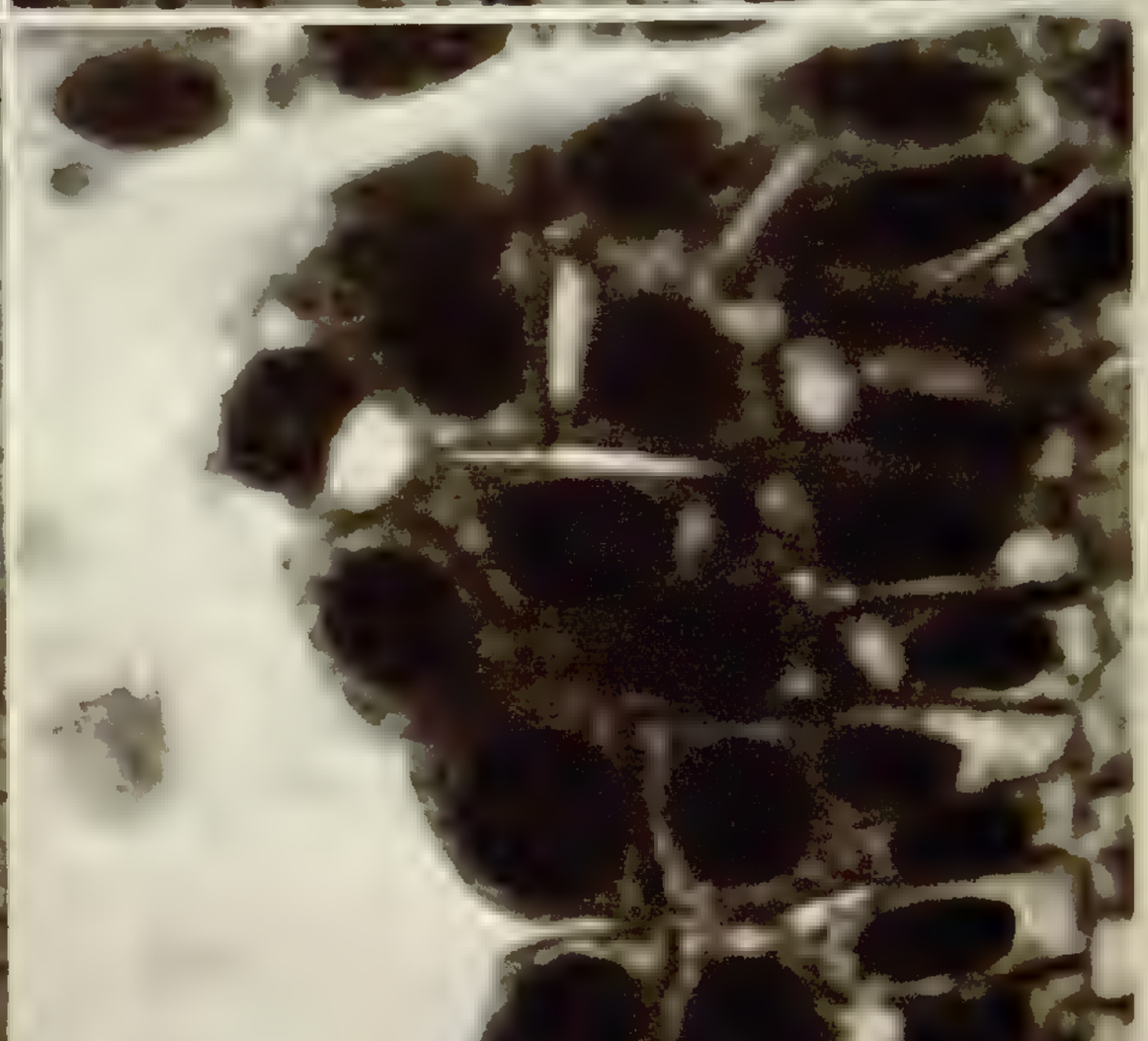
E. telmateia.—South side of Claremont Ave., just E of Alvarado St., Oakland, California, 4 Dec 1980 (Voucher: Hauke C1, KIRI).

Results

From the cleared material of *E. diffusum*, it was apparent that the sheath teeth enlarge and mature before there is much development of the lower portion of the sheath (Fig. 1). Such clearings also showed dramatically the mature form of the commissure, with its row of C-cells and oblique lines of neighboring cells (Figs. 2, 3). Longitudinal sections confirm what the clearings suggested, that the three youngest sheaths are primarily growing by tooth development, that the fourth and fifth youngest sheaths show rapid growth of the basal, fused part of the sheath and the commissures, and that the sixth and older sheaths have maturing or matured C-cell rows. The youngest sheaths do not show any median line of cells (Fig. 4). The earliest appearance of a median line is 4 large cells appearing approximately square in tangential sections (Fig. 5). The number increases until there are 10 or more square to slightly rectangular cells (Fig. 6). In cross-section of the sheath (Fig. 7) these cells can be seen as trapezoidal, so that an abaxial longitudinal section would appear square, whereas the adaxial section would appear rectangular. I could not determine whether the C-cell row originated by one or more of the pre-commissure sheath cells assuming a more median position, by longitudinal division of pre-commissure cells to create a median line of cells, or in some other manner. Once the row is present, the number of cells in it greatly increases by transverse division (Fig. 9) and the cells become more rectangular to tangentially elongated. That the increase in number of C-cells is by transverse division throughout the row rather than by basal initial activity is also indicated by the relation between the C-cells and the adjacent cells. At the cuboidal stage (Fig. 6), the two are about the same height. In the rectangular stage there are often two or more C-cells for each neighbor cell (Fig. 8). In the tangential stage, three or more C-cells may abut a single neighbor (Fig. 10).

Whereas the C-cells are dividing only transversely, the neighboring cells are dividing obliquely (Fig. 8, arrow) and thus establishing the oblique orientation of the adjacent cells. Adaxial to the C-cell row is an inner epidermis of vertically elongated cells. There may be two or more side-by-side under a given C-cell (Fig. 6, arrow), and each may be longer vertically than two C-cells (Figs. 11, 12). As the C-cells mature, they bend outward at the ends (Fig. 13) to achieve the shape of a letter C in transverse section. This bending causes a deep furrowing of the commissure in *E. diffusum* (Fig. 3).





FIGS. 1–3. *Equisetum diffusum*, clearings of sheaths. FIG. 1. Branch tip with young sheath showing precocious teeth elongation. Scale bar = 80 μm. FIG. 2. Commissure of C-cells and adjacent oblique cells. Scale bar = 50 μm. FIG. 3. Adjacent sheath segments with prominent commissural furrow. Scale bar = 200 μm. FIG. 4. *Equisetum diffusum*, tangential section, young sheath before initiation of C-cell row. The apex of the sheath is toward the left. Scale bar = 20 μm.

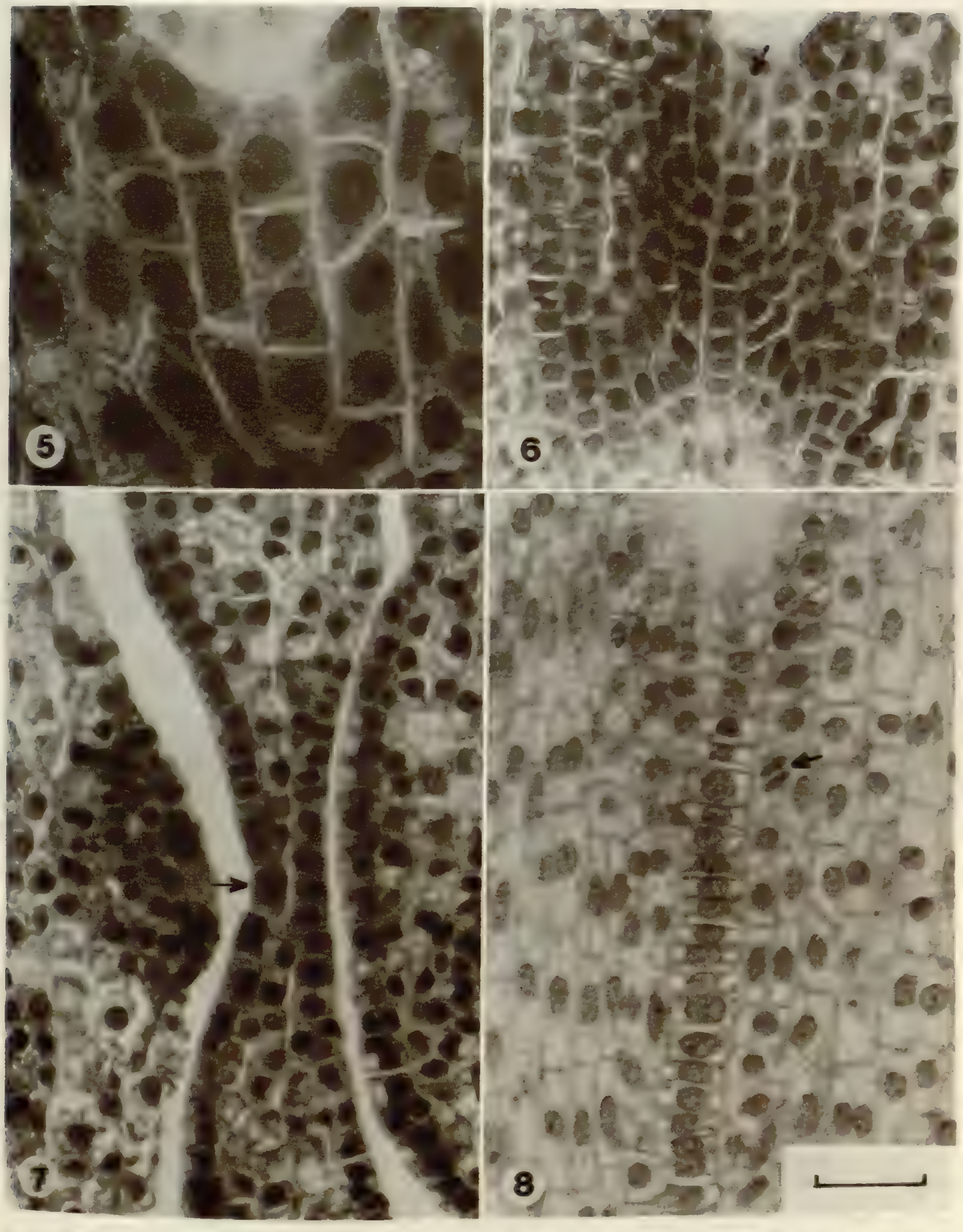
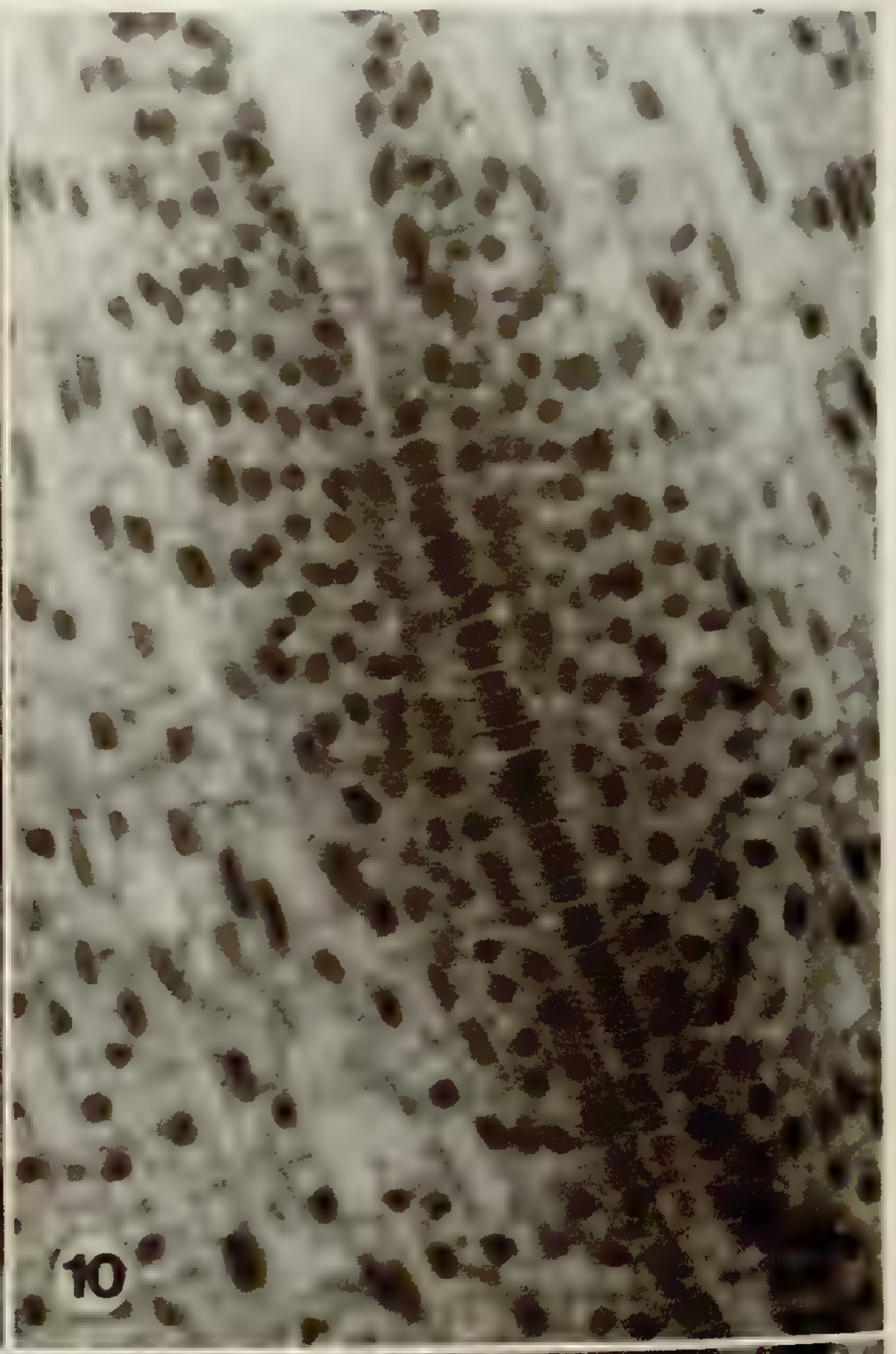
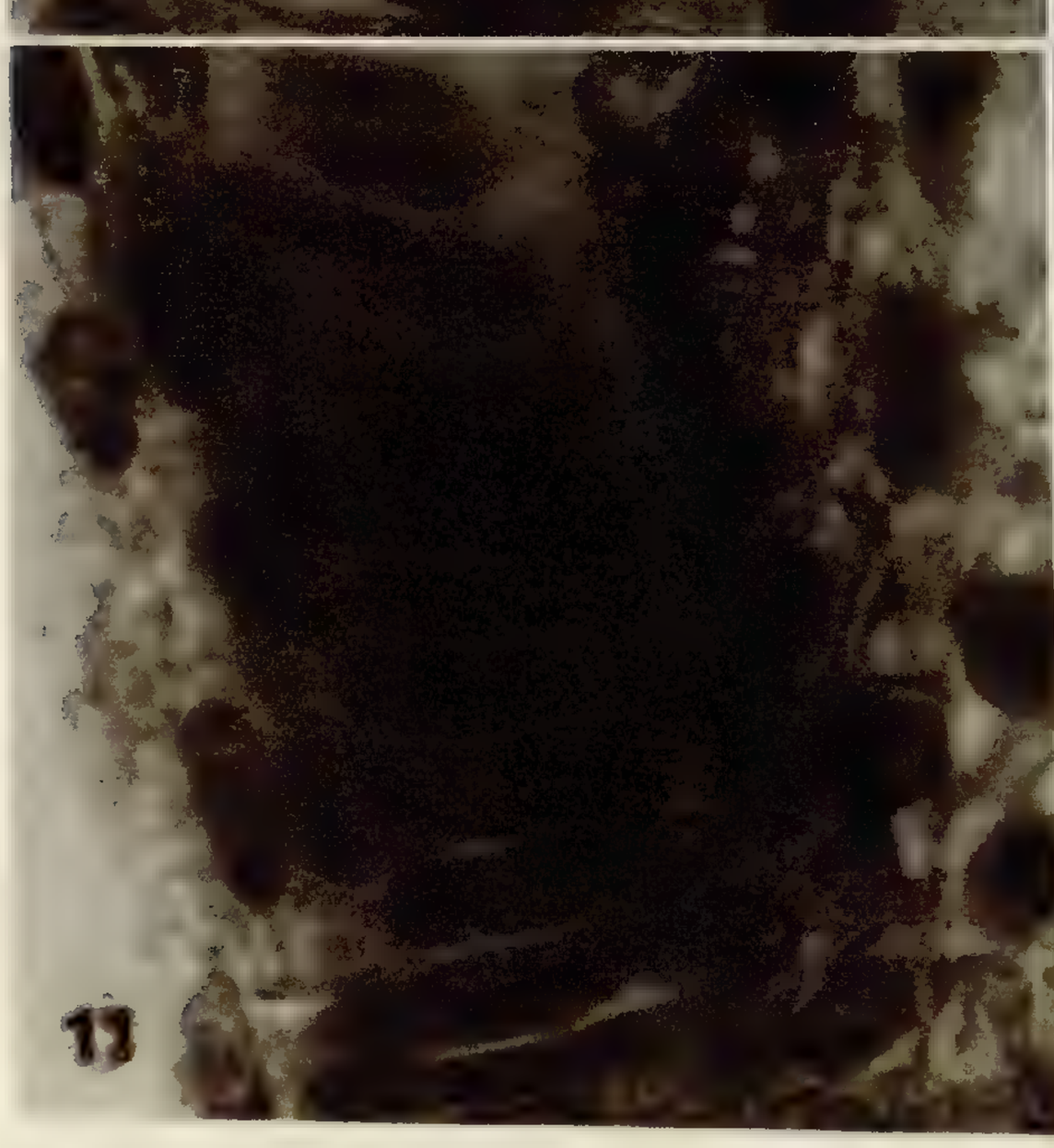


FIG. 5. *Equisetum diffusum*, tangential section, earliest visible C-cell row, of 4 cuboidal cells. Scale bar = 20 μm. FIG. 6. *Equisetum diffusum*, tangential section, C-cell row of more than 10 rectangular cells. Arrow indicates the underlying adaxial epidermal cells, taller than the C-cells and two abreast. Scale bar = 50 μm. FIG. 7. *Equisetum arvense*, cross-section, sheath with trapezoidal C-cell (arrow) underlain by adaxial epidermal cells. Scale bar = 50 μm. FIG. 8. *Equisetum telmateia*, tangential section, C-cell row of numerous rectangular cells. Arrow marks oblique division figure in a neighboring cell. Scale bar = 50 μm.





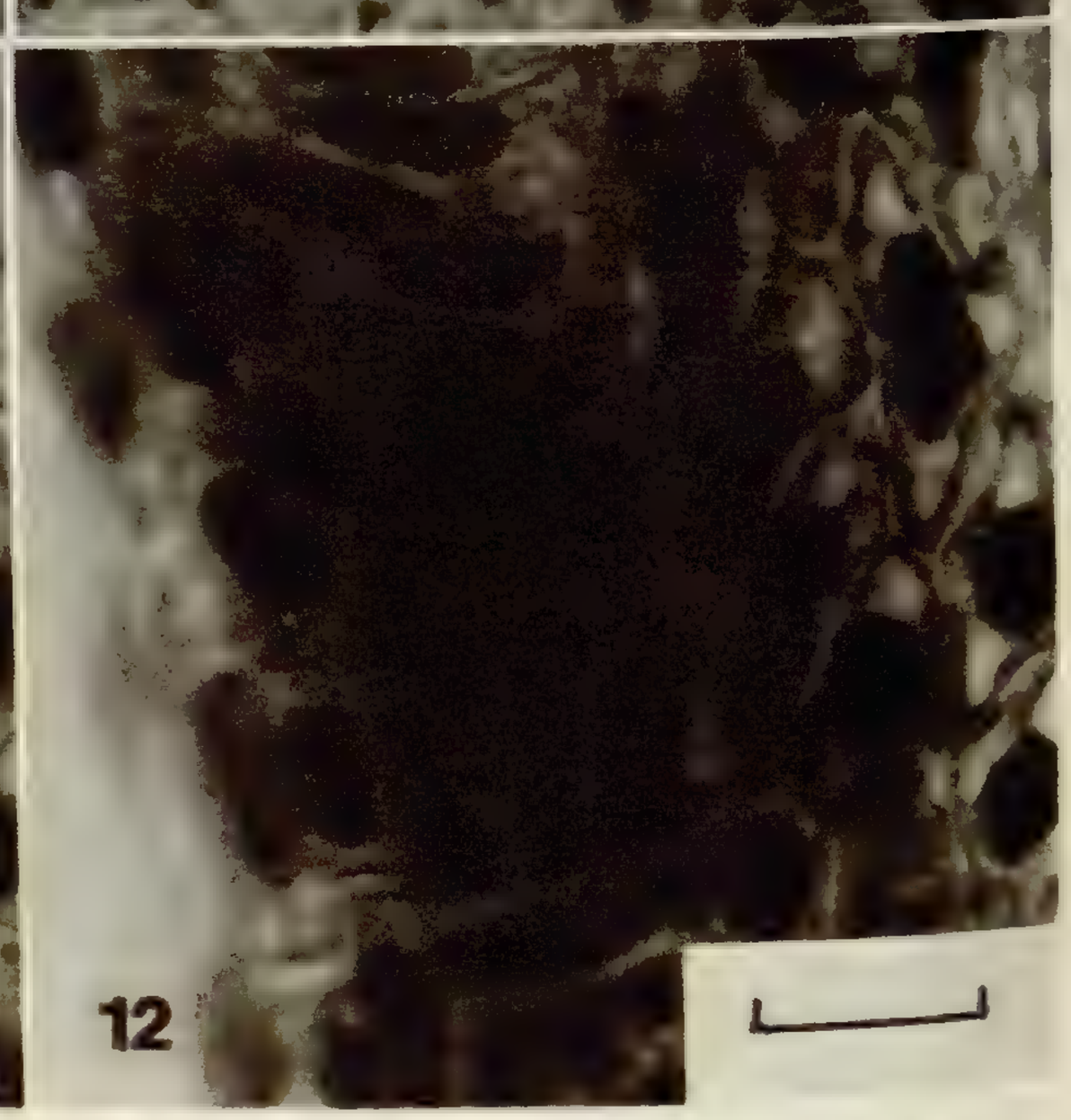
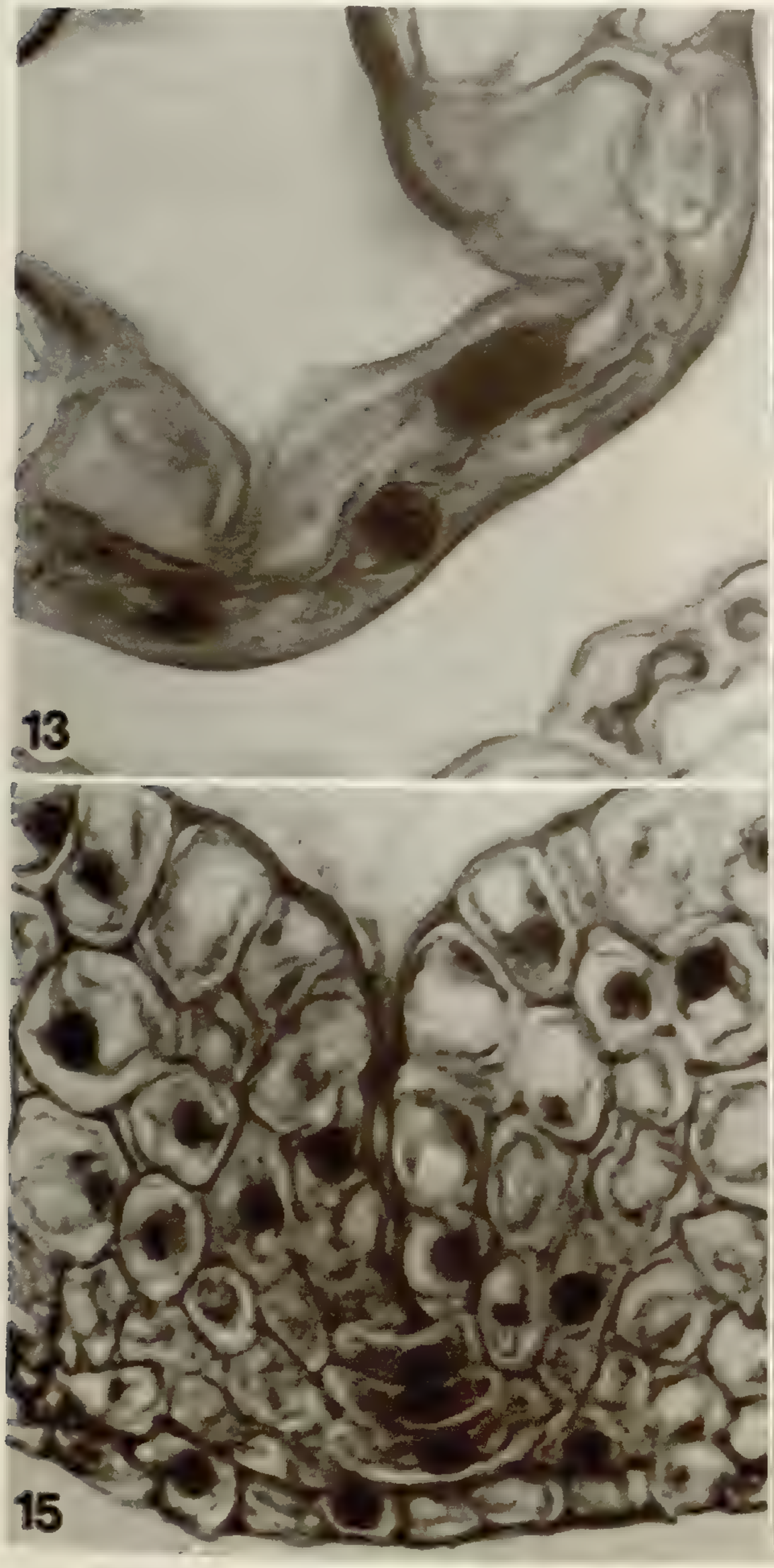


FIG. 9. *Equisetum telmateia*, tangential section, C-cell undergoing transverse division. Scale bar = 20 μm. FIG. 10. *Equisetum arvense* tangential section, commissure with C-cells tangentially elongated. Scale bar = 80 μm. FIGS. 11, 12. *Equisetum diffusum*, tangential section, two optical sections showing the C-cell row of tangentially elongated cells underlain by adaxial epidermal cells which are vertically elongated and narrower than the C-cells. Scale bar = 20 μm.



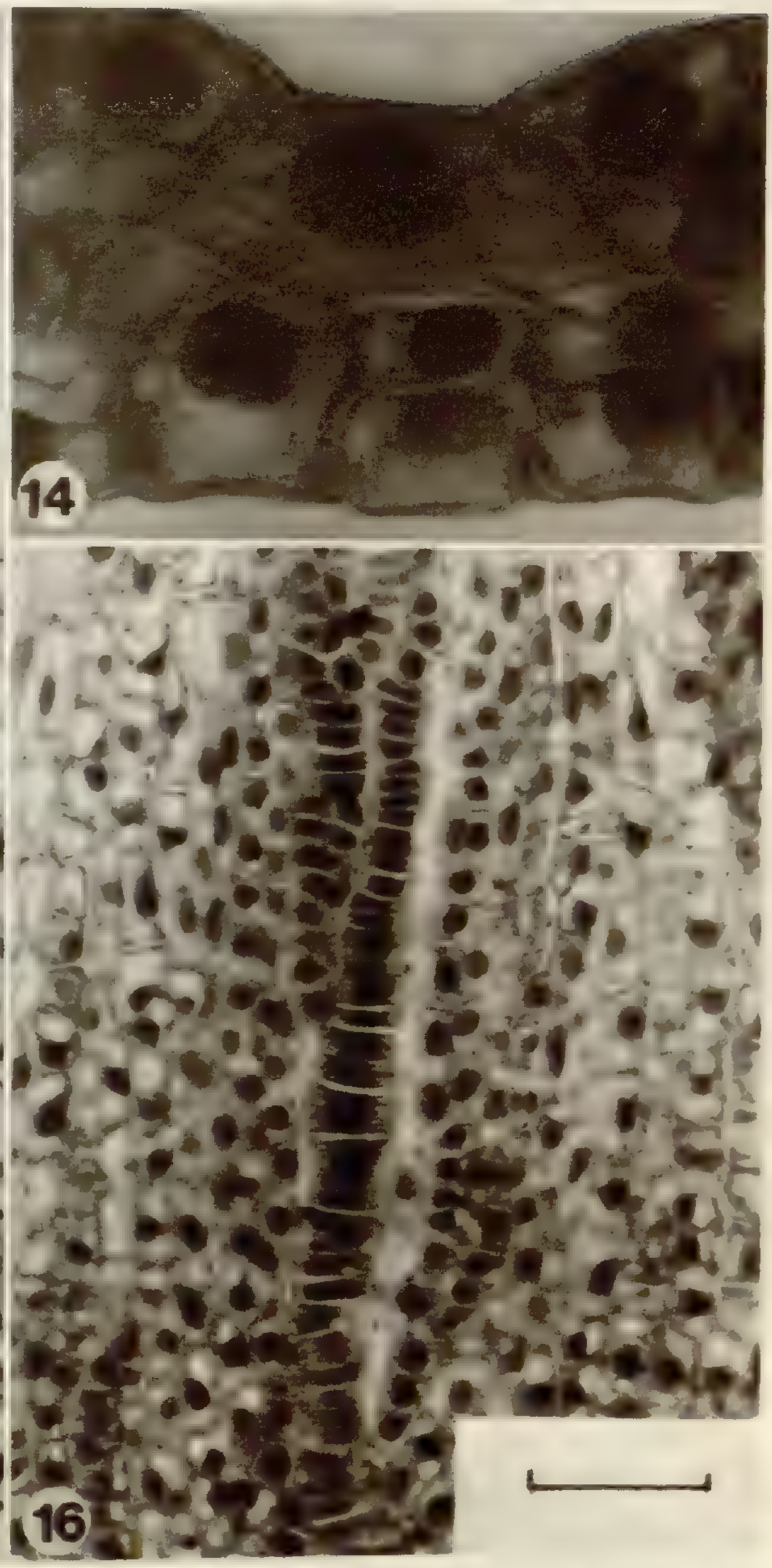


FIG. 13. *Equisetum diffusum*, cross-section, C-cell with prominently bent ends. Scale bar = 20 μm. FIG. 14. *Equisetum telmateia*, cross-section, commissure with adaxial epidermis proliferated by periclinal division. Scale bar = 20 μm. FIG. 15. *Equisetum hyemale*, cross-section, commissure of C-cells occluded by growth of neighboring cells. Scale bar = 50 μm. FIG. 16. *Equisetum diffusum*, tangential section, rhizome commissure showing less-ordered structure. Scale bar = 80 μm.

The ontogeny described here for the commissure of *E. diffusum* was also seen in *E. telmateia*. In that species, however, periclinal division of the adaxial layer (Fig. 14) in the basal part of the commissure made it multilayered. *Equisetum arvense*, also studied for comparison, does not develop as deeply furrowed a commissure as do the above two. Because of its occluded commissure, *E. hyemale* does not show the distinct C-cell row with oblique adjacent cells characteristic of *E. diffusum*, and was not studied developmentally. At maturity (Fig. 15) it does appear to be occluded by overgrowth of adjacent cells, as described by Müller (1888) and to have formed "pillow" cells by periclinal division of the C-cell.

The nucleus of the C-cell is particularly noteworthy. It is always prominent and remains in the center of the cell (Figs. 8, 9, 14). As the cell changes from cuboidal to rectangular to tangential, the nucleus becomes more extremely ellipsoidal. As the cell folds outward, the horizontally attenuated nucleus remains in the center portion (Fig. 13).

The distinctive commissures described are from aerial stems, or branches. The rhizome sheath commissures are apparently much less regular in their development (Fig. 16), for the C-cells do not form a single distinct, uniform row, nor do the adjacent cells form "chain lines."

Discussion

Although Müller's (1888) description of the commissure, with its distinctive median row of abaxial epidermal cells, the C-cells (his *ankerzellen*) was accurate, his "chain line" explanation for the oblique appearance of adjacent cells by stretching caused by growth of the sheath teeth, was not. Schellenberg (1895) attributed them to unequal growth at the base, which is more nearly correct. Actually, the characteristic appearance is the result of control of plane of division. In the median row, cell division is always transverse, and there is little subsequent elongation of daughter cells. Hence from a row of a few initially cuboidal cells is produced a longer row of rectangular cells, and eventually a very long row of tangentially elongate cells. This transverse division activity occurs throughout the C-cell row, and there is no basal initial, as Sadebeck (1902) implied. The cells adjacent to the commissural row divide in various plants, including obliquely, to produce the parabolic curves with C-cells at the apex. The distinctive outward bending of the C-cell, and the equally distinctive appearance and position of the nucleus cannot be explained here.

Since the sheath grows as a unit from an initial ring produced just below the apex, descriptions of the sheath segments as "concrescent," "fused," or "united" are misleading. These terms could be understood to indicate ontogenetic rather than phylogenetic fusion. The term "connate" is preferable, since it means literally "born together" and indicates congenital fusion.

Literature Cited

Bierhorst, D. W. 1971. *Morphology of vascular plants.* New York: The Macmillan Co.

Bold, H. C., C. J. Alexopoulos, and T. Delevoryas. 1980. *Morphology of plants and fungi.* 4th ed. New York: Harper & Row.

DeBlock, L. 1923. Contribution à l'étude des Equisétacées. Thesis. Faculty of Medicine & Pharmacy, Lille.

Duval-Jouve, J. 1864. *Histoire naturelle des* Equisetum *de France.* Paris: Bailliere et Fils.

Foster, A. S. 1934. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Tech. 9:91–92.

——— and E. M. Gifford. 1974. *Comparative morphology of vascular plants.* 2nd ed. San Francisco: Freeman.

Hauke, R. L. 1979. A taxonomic monograph of *Equisetum* subgenus *Equisetum.* Nova Hedwigia 30:385–455. (1978)

MILDE, J. 1867. Monographia Equisetorum. Nov. Actorum Acad. Caes. Leop.-Carol. German. Nat. Cur. 32(2):1–605 + 35 pl.

MÜLLER, C. 1888. Über den Bau der Commissuren der Equisetenscheiden. Jahrb. Wiss. Bot. 19: 497–575.

SADEBECK, R. 1902. Equisetaceae. Pp. 520–548 in *Die natürlichen Pflanzenfamilien*, Vol. 1(4). eds. A. Engler and K. Prantl. Leipzig: Verlag Engelmann.

SCHELLENBERG, H. C. 1895. Zur Entwicklungsgeschichte der Equisetenscheiden. Ber. Deut. Bot. Ges. 13:165–174.

SMITH, G. M. 1955. *Cryptogamic botany II. Bryophytes and pteridophytes*. 2nd ed. New York: McGraw Hill.

REVIEW

"*Ferns of Jamaica, a Guide to the Pteridophytes*," by George R. Proctor. 1985. vi + 631 pp. British Museum (Natural History). ISBN 0-565-00895-1. £50.00.

The publication of a complete new pteridophyte Flora for a tropical country is always a major event. Jamaica, being a small island (11,424 km^2; 4411 mi^2) with high mountains (up to 2250 m elevation) and varied topography and soils, supports an unusually large pteridophyte flora (609 taxa, 82 (13.3%) of them endemics) for its size. The *Ferns of Jamaica* is unique in that the author resided in Jamaica for many years. He made extensive field surveys and collections and saw almost all the taxa as living plants. This contrasts greatly with other Floras, which are by necessity based largely on herbarium specimens with occasional trips to the field by the authors. Thus, the habitat data given by Proctor are unusually complete and helpful, and even serve to amplify the distinctions between closely related species like *Pityrogramma williamsii* Proctor (on calcareous earth banks and limestone boulders) from the similar appearing *P. sulphurea* (Swartz) Maxon (on non-calcareous earth banks). The well-written keys, brief descriptions, and habitat and frequency statements make this Flora easy and pleasant to use; one could read it cover-to-cover for the pleasure of discovering the many original ecological observations. Some of the species of each genus are illustrated, often by reproducing graceful line drawings found in 19th century works on ferns. The taxonomic concepts are broad at all levels, but subcategories are freely employed. The book is the capstone and monument to the productive years that Proctor spent in Jamaica, but it is by no means a culmination: he is currently preparing a Flora of the pteridophytes of Puerto Rico and the Virgin Islands, to which he has added dozens of species new to the Flora as a result of intensive fieldwork and a consummate knowledge of Antillean Pteridophyta.—DAVID B. LELLINGER, Department of Botany, National Museum of Natural History NHB-166, Smithsonian Institution, Washington, DC 20560.

American Fern Journal 75(4):120–127 (1985)

Wild Gametophytes of *Equisetum sylvaticum*

JEFFREY G. DUCKETT
School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS, U.K.

Though generally stated to be rare in nature, large populations of *Equisetum* gametophytes can sometimes be found on bare mud on the exposed margins of lakes and rivers in Britain. A detailed analysis of the reproductive biology and dynamics of such wild populations is given by Duckett and Duckett (1980). Apart from these littoral sites, gametophytes are occasionally seen on bare damp soil by field bryologists (Appleyard, 1981; Crundall, 1983). Elsewhere in the world *Equisetum* gametophytes have been recorded on bare mud, particularly that exposed on river banks, following the monsoon in the Indian subcontinent (Mohan-Ram & Chatterjee, 1971) or seasonal rains and the melting of winter snow in North America (Walker, 1921, 1931, 1937; Mesler & Lu, 1977; Duckett & Duckett, 1980).

The frequent occurrence of hybrid horsetails with distributions indicative of multiple origin, rather than fragmentation, and long distance dispersal of single clones (Duckett, 1979), indicates that sexual reproduction in this group of pteridophytes may not be as infrequent as normally assumed. The apparent rarity of the gametophytes is related to the production of short lived spores over a short period during each growing season (Duckett, 1970a) and narrow habitat tolerance in situations which only appear sporadically: bare mud which is neither too dry nor too wet must be available for colonization when the spores are liberated. Even after establishment the gametophytes are poor competitors with bryophytes and vascular plants and are apparently excluded from the immediate vicinity of the parent plants by allelopathic compounds (Milton & Duckett, 1985).

Detailed scrutiny of the literature on wild gametophytes reveals that the majority of observations have been made on very few species. Only those of *E. arvense* L., *E. palustre* L. and *E. fluviatile* L. have been found in Britain. I have failed to find those of *E. telmateia* Ehrh. and *E. variegatum* Schleich ex Weber and Mohr despite intensive search on many occasions of apparently suitable habitats in areas of water seepage and in wet depressions in sand dunes respectively. Attempts to establish wild gametophytes of *E. telmateia* on carefully marked sites where spores had been sown artificially were also unsuccessful (J. N. B. Milton, unpubl. data).

North American records are mainly for species in subgenus *Hippochaete* [*E. laevigatum* A.Br. (Walker, 1921, 1931), *E. scirpoides* Michx. (Walker, 1937; Feigley, 1949) and *E. hyemale* (Mesler & Lu, 1977)] plus records for *E. arvense* (Walker, 1931; Matzke, 1941; Hauke, 1967) and a single locality with *E. telmateia* (Walker, 1931). Descriptions from the Indian subcontinent are all referable to *E. ramosissimum* Desf. (Mohan-Ram & Chatterjee, 1971).

Wild gametophytes of *E. pratense* Ehrh. have never been described and the only possible record for *E. sylvaticum* L. dates from Bischoff (1853). From the description provided, plus the lack of morphological information on cultured

gametophytes at that time it is far from certain that these gametophytes can be referred to *E. sylvaticum*.

Whilst collecting bryophytes in late August 1984 on the normally inundated margin of the southern shore of Loch an Daimh, Glen Lyon, Mid West Perthshire, Scotland, Professors R. Brown, B. Lemmon (University of Louisiana) and I discovered a population of *Equisetum* gametophytes. Since *E. sylvaticum* was the only horsetail present in abundance in the vegetation surrounding Loch an Daimh, and the gametophytes differed in sexuality from those of other species of *Equisetum* previously seen in Britain, it seemed likely that the wild gametophytes were also of this species. Identification was subsequently confirmed from comparison with *E. sylvaticum* gametophytes grown in axenic culture.

This paper compares the morphology and sexual behavior of the wild gametophytes of *E. sylvaticum* with (1) gametophytes of *E. sylvaticum* in axenic culture (2) wild gametophyte populations of other species of *Equisetum*. Features of the *E. sylvaticum* habitat are compared with those of other wild gametophyte localities.

Nomenclature follows Corley and Hill (1981) for bryophytes and Clapham, Tutin, and Warburg (1962) for vascular plants.

Observations

The site.—Loch an Daimh is a reservoir constructed in the 1950s in the upper part of Glen Lyon. The elevation of the high water level is approximately 400 meters. In August 1984 the water was some 10 meters below this, having fallen steadily since the spring (D. Long, pers. comm.) during one of the driest seasons on record in the Scottish Highlands.

The vegetation bordering the south shore of the Loch is referable to the Callunetum vulgaris, Vaccineto-Callunetum and Molinieto-Callunetum associations of McVean and Radcliffe (1962), but with the addition of abundant *Equisetum sylvaticum* (20–50 stems per m^2). A few stems of *E. palustre* and *E. fluviatile* were found by the outflow stream below the dam and *E. variegatum* and *E. arvense* grew in small quantities on the sides of streamlets entering the Loch. In comparison with *E. sylvaticum* it is unlikely that these four species make a significant contribution to the spore rain.

The *Equisetum* gametophytes were restricted to a layer of sandy peat, pH 5.2, about 1 meter above the low water level along a 200 meter stretch of shore from grid reference 27/507462 to 27/502464. They were growing on both horizontal and vertical surfaces of the peat and around the remains of *Calluna* plants which predated the construction of the dam. In the immediate vicinity of the gametophytes only about 5% of the surface of the reservoir margin was covered with vegetation.

In consisting of a normally inundated reservoir margin, this site closely resembles other localities in Britain where *Equisetum* gametophytes are of regular occurrence. However, with one exception (Ladybower, Yorkshire with a value of 5.5) the pH at Loch an Daimh (pH 5.2) was significantly lower than at the other sites (6.0–7.5). The gametophytes were associated with 8 species of pha-

nerogams (highly depauperate compared with up to 80 at other sites), 23 liverworts, and 39 mosses. Most abundant were *Festuca ovina, Isolepis setacea, Pellia epiphylla, Jungermannia gracillima, Pogonatum aloides, Ditrichum heteromallum, Dicranella subulata, D. rufescens, Campylopus paradoxus, Ephemerum serratum*, var. *serratum* and *Pohlia nutans*. These species are indicative of a substrate of much lower nutrient status than that of Pennine reservoirs in Northern England (Duckett & Duckett, 1980; Duncan & Dalby, 1960). These observations on pH and associates suggest that the gametophytes of *E. sylvaticum* may have different ecological requirements from those of other horsetails.

Gametophyte morphology and sexual behavior.—The gametophytes of *E. sylvaticum* were more or less circular cushions varying from 0.5–2.0 mm in height and dark, dull green in color. They ranged in diameter from 2–9 mm (mean 4.7 mm). All the 49 individuals collected were female. None bore sporophytes.

The overall morphology of the gametophytes was identical to that of other species in the wild. Each comprised a basal cushion 10–20 cells thick bearing abundant colorless rhizoids ventrally [the morphologically similar *Fossombronia fimbriata*, also found at Loch an Daimh, has purple rhizoids (Paton, 1974)] and closely packed photosynthetic lamellae on the dorsal surface (Fig. 1). Archegonia (Fig. 2) are produced by the marginal meristem of the cushion and at maturity lie at the base of the lamellae.

Earlier attempts (Duckett, 1970, 1973) at growing spores of *E. sylvaticum* resulted solely in production of male gametophytes. However, by using Parker medium (Klekowski, 1969) on which gametophytes grow more rapidly than on the Knop and Beijerinck agars previously employed, a sexual behavior pattern similar to that in all other species of the subgenus *Equisetum* is produced. Identical results to those set out in Fig. 3 for spores from North America (Deerfield, Massachusetts) have also been obtained from British material.

The majority of the spores grow into male gametophytes which produce antheridia indefinitely. The remainder are initially female but subsequently produce antheridia. Following flooding of the latter, sporophytes (from 1–6 per gametophyte) are produced by self-fertilization.

Assuming that the wild gametophytes at Loch an Daimh derived from spores germinating in May, when collected in late August they would be approximately 125 days old. Thus their sexuality is very different from that of cultured gametophytes. Aside from the predominance of males, in culture virtually all the females have subsequently produced antheridia by this time.

It is noteworthy that Rumberg (1932), using mineral nutrient agars as culture medium, found that female gametophytes of *E. sylvaticum* subsequently produced antheridia at much the same rate as recorded here (Fig. 3), whereas those grown by Buchtien (1887) on sand and soil remained female.

The salient features of the lamellae and archegonia of *E. sylvaticum* are illustrated in Figures 1 and 2. A detailed description of the antheridia is given by Duckett (1973). Lamellae in both the cultured and wild individuals of *E. sylvaticum* are of plate type characteristic of subgenus *Equisetum* (Duckett, 1973) and as in other species, vary greatly in size and shape. However, examination of a large number reveals that apices which are obtusely lobed and flat predominate

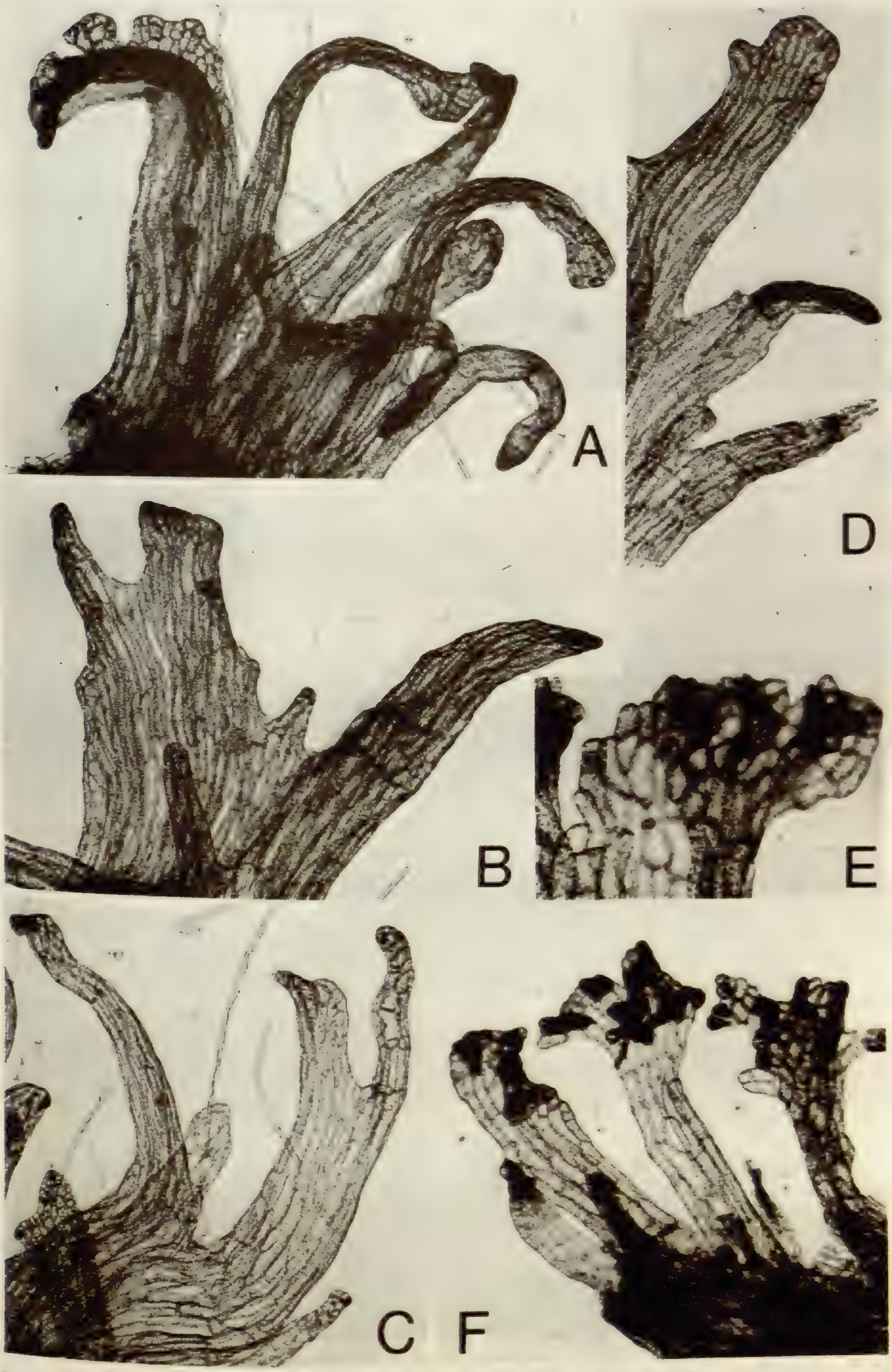


FIG. 1. Lamellae from female gametophytes of *Equisetum sylvaticum*. A–C from cultured gametophytes. D–F from wild gametophytes. All × 35 except, E, × 100.

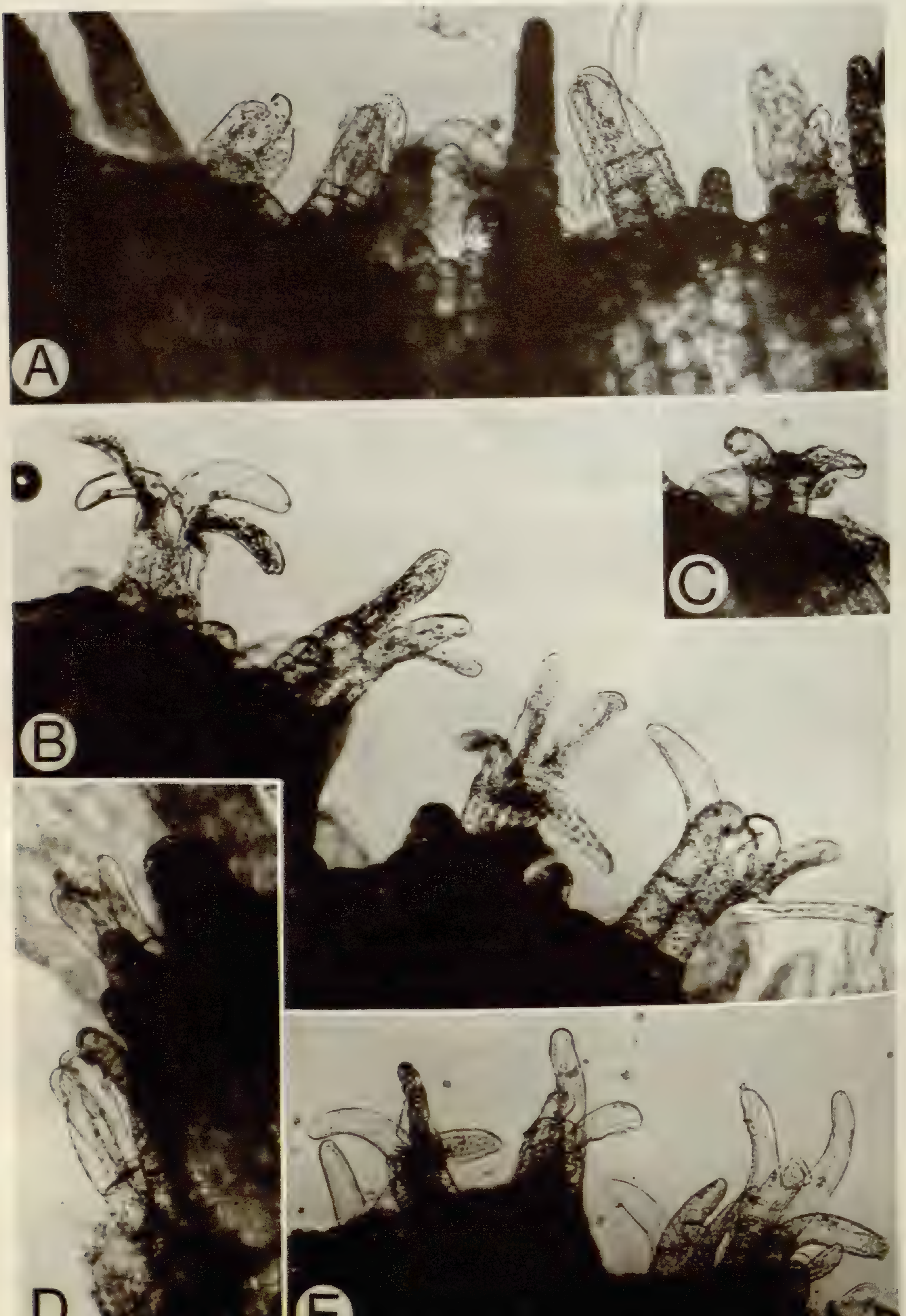


FIG. 2. Archegonia of *Equisetum sylvaticum*. A, B from cultured gametophytes. C–E from wild gametophytes. All × 100.

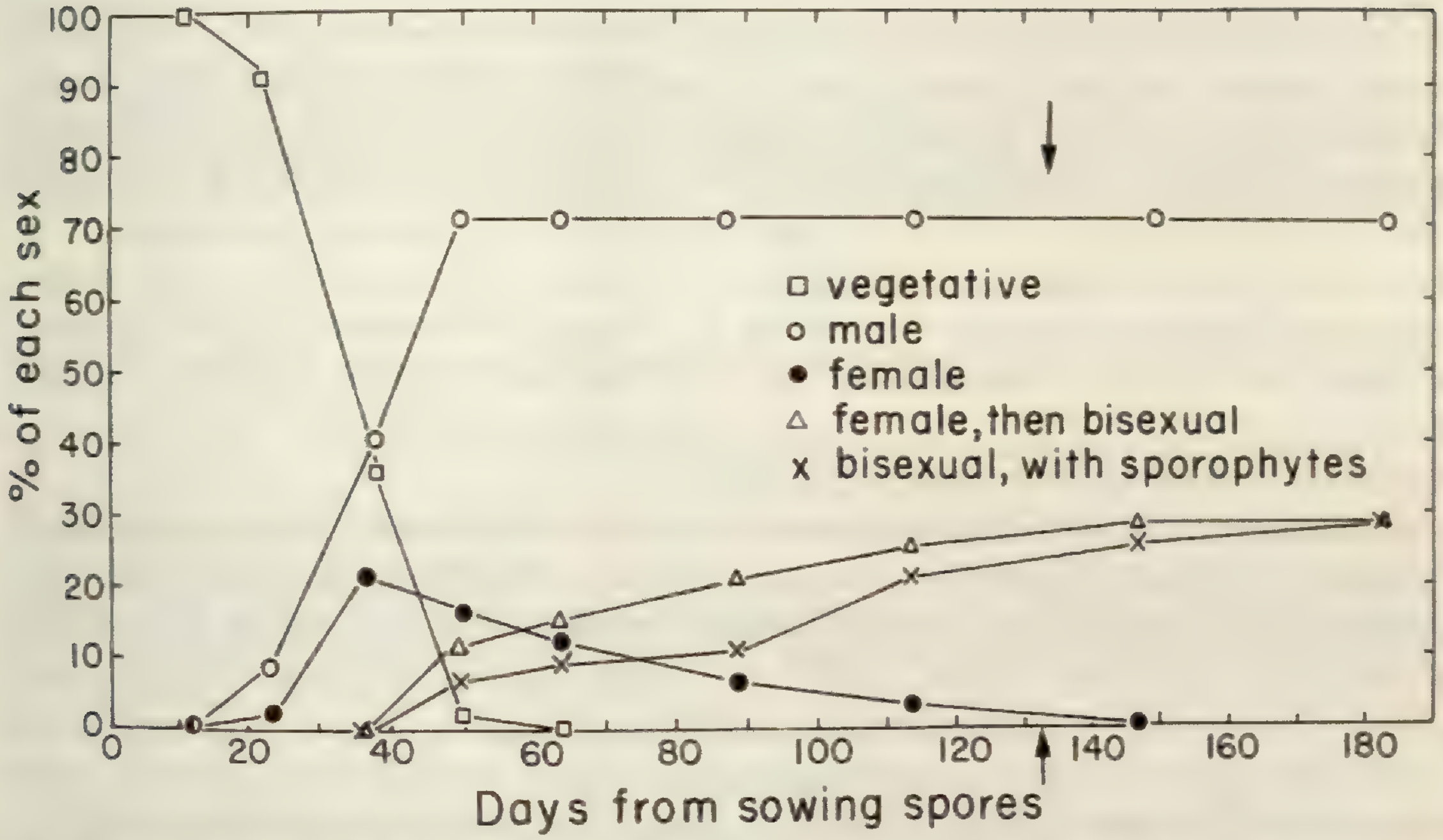


FIG. 3. Sexual behavior of gametophytes of *Equisetum sylvaticum* grown in single spore cultures on Parker medium (Klekowski, 1969). 300 spores, collected from Deerfield, Massachusetts, were cultured as set out in Duckett (1970). Arrows indicate the approximate age of the wild gametophytes.

(cf. the funnel-shaped apices of *E. arvense*). The apical cells are usually short whereas those in *E. telmateia* and *E. fluviatile* are elongate. In gross morphology the lamellae of *E. sylvaticum* are closest to those of *E. palustre*. The latter species however possesses apical cells that are usually less than 30–40 μm wide whereas those of *E. sylvaticum* mostly fall into the size range 30–60 μm.

In common with other members of the genus, archegonia of *E. sylvaticum* possess protruding necks composed of three tiers, each of four cells. At maturity the cells of the terminal tier elongate considerably (up to six times their original length), separate from each other, and bend away from the neck canal. This maturational divergence appears to be more variable in *E. sylvaticum* than in other species of *Equisetum*. Prior to divergence the terminal neck cells are slightly twisted both in cultured (Fig. 2A) and wild (Fig. 2D) specimens. The subterminal neck cells of *E. sylvaticum* are much shorter than those of *E. fluviatile* and *E. arvense* (Duckett & Duckett, 1980).

DISCUSSION

On the basis of morphology, sexuality, and availability of spores in the adjacent vegetation, there seems to be little doubt that the wild gametophytes from the shore of Loch an Daimh are referable to *E. sylvaticum*. Although the gross morphology of these wild gametophytes is the same as that of other species in nature, they differed in two major respects; sexuality and the absence of sporophytes.

Wild gametophyte populations of *E. arvense*, *E. palustre*, and *E. fluviatile* in

late spring comprise mixtures of male and female individuals. But, by the end of each season few or no males can be found. However, the presence of sporophytes on the vast majority of the females which remain attests to their earlier occurrence. Thus it would seem likely that no male gametophytes of *E. sylvaticum* were ever present at Loch an Daimh in 1984. It may also be significant in this context that the wild gametophytes of *E. sylvaticum* (mean diameter 4.7 mm) were significantly larger than females of *E. arvense*, *E. fluviatile*, and *E. palustre* (all less than 3 mm) of comparable age. This suggests that conditions were particularly favorable for the growth of *E. sylvaticum* gametophytes, a situation that would act against the production of males.

On the basis of size data now available for *E. arvense*, *E. sylvaticum*, *E. fluviatile*, *E. palustre*, and *E. telmateia* (Walker, 1931) it appears that wild gametophytes of species in subgenus *Equisetum* rarely if ever achieve dimensions of between 10 mm and 35 mm, recorded in *Hippochaete* (Feigley, 1949; Mohan-Ram & Chatterjee, 1971; Mesler & Lu, 1977).

Although the occurrence of wild gametophytes described here did not result in production of sporophytes of sexual origin, this incident establishes the potential for sexual reproduction in *E. sylvaticum*.

At some similar site, but where *E. pratense* grows alongside *E. sylvaticum* one may readily envisage the origin of *E.* ×*mildeanum* Rothm., the hybrid involving the two species in subsection *Subvernalia* (Duckett, 1979). When grown axenically under identical culture regimes each species of *Equisetum* has a distinct pattern of sexual behavior (Duckett, 1970, 1972). Specific differences in the proportions of males and females in culture are paralleled in mixed species gametophyte populations found in the wild. Two hybrid horsetails, *E. arvense* × *E. fluviatile* and *E. fluviatile* × *E. palustre* have already been found to arise from such situations (Duckett & Duckett, 1980). Under the culture regime used in this investigation 27% of the spores of *E. sylvaticum* produced female gametophytes but the corresponding figure for *E. pratense* was only 8% (Duckett, unpubl. data). A similar difference in sexuality in the wild would clearly favor the production of hybrid sporophytes.

Literature Cited

Appleyard, J. 1981. The annual meeting, 1980, Bristol. Bull. Brit. Bryol. Soc. 37:7–14.

Bischoff, G. W. 1853. Bermerkungen zur Entwicklungsgeschichte der *Equisetum*. Bot. Zeitung (Berlin) 11:97–109.

Buchtien, O. 1887. Entwicklungsgeschichte des Prothallium von *Equisetum*. Biblioth. Bot. 2:1–49.

Clapham, A. R., T. G. Tutin, and E. F. Warburg. 1962. *Flora of the British Isles*. 2nd ed. Cambridge: Cambridge University Press.

Corley, M. F. V. and M. O. Hill. 1981. *Distribution of bryophytes in the British Isles. A census catalogue of their occurrence in Vice Counties*. Cardiff: British Bryological Society.

Crundall, M. L. 1983. The summer meeting, 1982, Penrith. Bull. Brit. Bryol. Soc. 41:5–8.

Duckett, J. G. 1970. Sexual behaviour of the genus *Equisetum*, subgenus *Equisetum*. J. Linn. Soc., Bot. 63:327–352.

———. 1972. Sexual behaviour of the genus *Equisetum*, subgenus *Hippochaete*. J. Linn. Soc., Bot. 64:87–108.

———. 1973. Comparative morphology of the gametophytes of the genus *Equisetum*, subgenus *Equisetum*. J. Linn. Soc., Bot. 66:1–22.

———. 1979. An experimental study of the reproductive biology and hybridization in the European and North American species of the genus *Equisetum*. J. Linn. Soc., Bot. 79:205–229.

——— and A. R. DUCKETT. 1980. Reproductive biology and population dynamics of wild gametophytes of *Equisetum*. J. Linn. Soc., Bot. 80:1–40.

DUNCAN, J. E. AND M. DALBY. 1960. The vegetation of Swinsty and Fewston reservoirs 1957 to 1959. Naturalist, Hull 874:81–88.

FEIGLEY, M. 1949. An occurrence of gametophytes of *Equisetum* in Cheboygan County, Michigan. Amer. Fern J. 39:106–109.

HAUKE, R. L. 1967. Sexuality in a wild population of *Equisetum arvense* gametophytes. Amer. Fern J. 57:59–66.

KLEKOWSKI, E. Jr. 1969. Reproductive biology of the Pteridophyta. III. A study of the Blechnaceae. J. Linn. Soc., Bot. 62:361–377.

MCVEAN, D. N. and D. A. RATCLIFFE. 1962. *Plant communities of the Scottish Highlands. A study of Scottish mountain moorland and forest vegetation*. London: Her Majesty's Stationery Office.

MATZKE, E. B. 1941. Gametophytes of *Equisetum arvense*. Torreya 41:181–187.

MESLER, M. R. and K. L. LU. 1977. Large gametophytes of *Equisetum hyemale* in northern California. Amer. Fern J. 67:97–98.

MILTON, J. N. B. and J. G. DUCKETT. 1985. Potential allelopathy in *Equisetum*. Proc. Roy. Soc. Edinburgh 86:468–469.

MOHAN-RAM, H. Y. and J. CHATTERJEE. 1970. Gametophytes of *Equisetum ramosissimum* subsp. *ramosissimum*. II. Sexuality and its modification. Phytomorphology 20:151–172.

PATON, J. A. 1974. *Fossombronia fimbriata* sp. nov. J. Bryol. 8:1–4.

RUMBERG, J. 1932. Entwicklungsgeschichte der Prothallien von *Equisetum sylvaticum* und *E. palustre*. Planta 15:1–42.

WALKER, E. R. 1921. The gametophytes of *Equisetum laevigatum*. Bot. Gaz. (Crawfordsville) 71:378–391.

———. 1931. The gametophytes of three species of *Equisetum*. Bot. Gaz. (Crawfordsville) 92:1–22.

———. 1937. The gametophytes of *Equisetum scirpoides*. Amer. J. Bot. 24:40–43.

American Fern Journal 75(4):128–132 (1985)

Osmunda cinnamomea forma *frondosa* at Mountain Lake, Virginia

CHARLES R. WERTH
Department of Botany, University of Kansas, Lawrence, KS 66045
MELANIE L. HASKINS
Department of Biology, University of Richmond, Richmond, VA 23173
AKKE HULBURT
Mountain Lake Biological Station, University of Virginia, Pembroke, VA 24136

Pronounced dimorphism of sterile and fertile pinnae is a conspicuous morphological attribute of all species of *Osmunda*. Sporangia are borne on the margins of highly reduced segments that lack chlorophyll at maturity. The green spores initially give these fertile pinnae a greenish cast, but after release of the spores, they become wholly cinnamon-brown or gray-brown and soon wither. Of the three *Osmunda* species occurring in eastern North America, two bear fertile pinnae on otherwise undifferentiated sterile fronds: in Royal Fern, *O. regalis* L., these fertile pinnae occur at the leaf tip, while in the Interrupted Fern, *O. claytoniana* L., two to six fertile pinnae occur in the central portion of the frond. In the Cinnamon Fern, *O. cinnamomea* L., the dimorphism is more complete, as sporophylls consist entirely of fertile pinnae and become completely achlorophyllous.

Upon our arrival at Mountain Lake Biological Station in the summer of 1984, we were surprised to discover a large number of *O. cinnamomea* plants bearing partially fertile fronds. These ranged continuously from those which were almost normal, i.e., with only a small portion of sterile blade tissue (usually at the tip) to those which were almost totally sterile, bearing only a few fertile segments, or even simply a few sporangia on sterile segment margins. The distribution of fertile pinnae on the fronds was highly irregular and unpredictable. The most frequent pattern was that with the lower portion of the frond fertile and intergrading into an upper sterile portion (Fig. 1A, B), but numerous fronds were found with a reverse pattern (Fig. 1C), or with sterile pinnae both above and below fertile ones (Fig. 1D). Numerous specimens were taken and have been deposited in the University of Richmond Herbarium.

By midsummer, normal fertile fronds of *O. cinnamomea* wither and fall to the ground or lie draped over sterile leaves. However, the sterile portions of the intermediate leaves remained turgid throughout the summer, making them especially conspicuous (a few of the nearly completely fertile fronds withered). This allowed us to take note of their distribution during our travels in the Mountain Lake area (Giles and Montgomery counties, Virginia) in the summers of 1984 and 1985. It became apparent that the intermediate fronds were very localized; we found them only in the immediate vicinity of the Biological Station and on Bald Knob and Bean Field Mountain near Mountain Lake (about 2 km southwest of the Biological Station). In these areas were patches where the in-

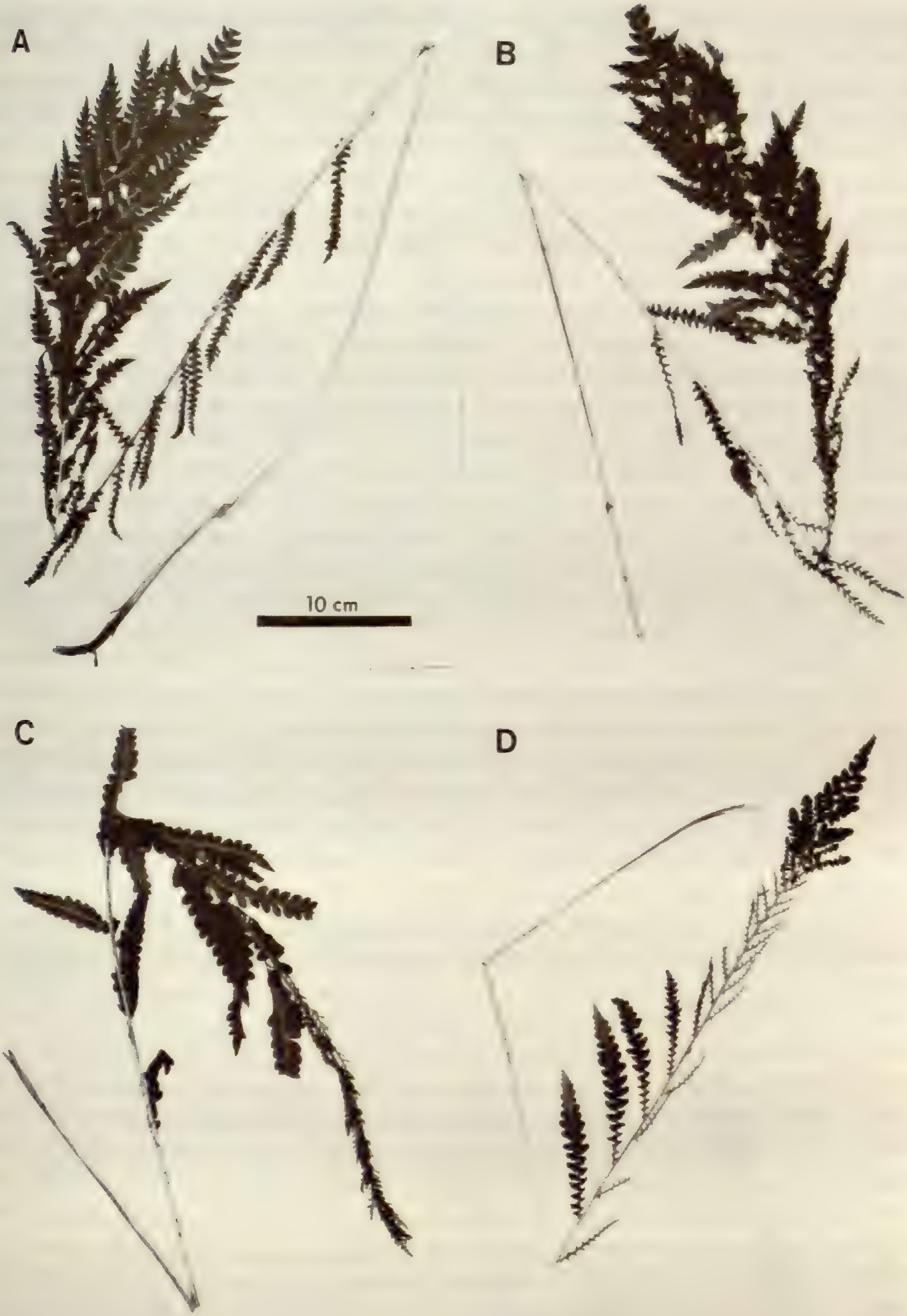


FIG. 1. *Osmunda cinnamomea* forma *frondosa* sporophylls collected at Mountain Lake in the summer of 1984, showing a portion of the range of variation in distribution of fertile pinnae. A and B. Most frequent variant with predominantly fertile lower portion intergrading into predominantly sterile upper portion. C. Leaf with sterile base and fertile tip. D. Leaf with fully fertile area in middle of frond, and with one side more fertile than other.

termediate fronds predominated, although normal sporophylls were always found with them, sometimes on the same plant. Virtually all leaves produced during the summer of 1985 were normal, although one fertile-tipped intermediate was noted on the Biological Station grounds.

Plants with such leaves were originally accorded varietal status as *O. cinnamomea* var. *frondosa* (Torrey, 1840), but the later reduction to forma (Britton, 1890) represents a much more satisfactory treatment. This leaf form is an environmentally induced abnormality and in this respect is analogous to numerous other trophophyll-sporophyll intermediates known in normally dimorphic ferns such as *Onoclea sensibilis* forma *obtusilobata* (discussed by Beitel et al., 1981).

In some ferns with dimorphic or subdimorphic fronds, production of intermediate fronds is frequent or even characteristic. For example in *Asplenium platyneuron*, intermediate leaves can be found on most individuals. Developmental control appears to be exceedingly rigid in *O. cinnamomea*, however, since forma *frondosa* has rarely been reported (Torrey, 1840; McLouth, 1897; Owen, 1901; House, 1933; Kittredge, 1941; Margaret Wren, pers. comm.; Murray Evans, pers. comm.). The cause of this rare phenomenon has never been fully explained, despite its possible significance in understanding the reproductive biology and/or morphogenesis of leaves in *O. cinnamomea*. A complete explanation will require further study, but for the present the following observations are relevant.

It is quite possible that production of sporophylls in *O. cinnamomea* is dependent upon an elevated nutritional level in the shoot apex. Production of sporophylls in nature is well known to occur primarily in open areas or under partial canopies. Enhanced sucrose concentrations favor development of excised leaf primordia into sporophylls *in vitro* (Sussex & Steeves, 1958; Harvey & Caponetti, 1972, 1973). Also, presence of ammonium ion is required for *in vitro* formation of fertile leaves (Harvey & Caponetti, 1974).

The expression of *frondosa* leaves often seems to be linked to environmental trauma (similarly to formation of partially fertile *Onoclea sensibilis* fronds—see Beitel et al., 1981). McLouth (1897) found *frondosa* fronds emerging in the fall from plants whose leaves had been destroyed by fire. House (1933) reported finding *frondosa* leaves in July on plants surrounded by road tar, though it is not made clear whether the tar had been applied the same or the previous summer. Owen (1901) reported *frondosa* leaves following a late spring frost. Margaret Wren (pers. comm.) collected *frondosa* in the fall of 1980 *and* in the spring of 1981 at a road construction site in Myrtle Beach, South Carolina, which had been disturbed by Hurricane David in the fall of 1979. Leaves are determined as being sterile or fertile at the latest by the summer preceding their emergence (Steeves & Wetmore, 1953). It is reasonable to hypothesize that injury to trophophylls, which would lower nutrient flow to the shoot apex, might effect a partial reversal in the determination of developing leaf primordia. These altered leaves would then emerge late in the same season or the following spring.

Steeves (1959) hypothesized that a reversal from sterile to fertile condition resulted in the predominantly fertile-tipped fronds observed by himself and by

Torrey (1840), based on the assumption that determination is directed from base to tip of the leaf primordium (Steeves & Briggs, 1958). Extending this hypothesis would lead us to predict that the leafy-tipped fronds observed by us and by Kittredge (1941) resulted from a reversal from fertile to sterile. However, the highly varied distribution of fertile pinnae we observed casts some doubt on this simplified explanation. It is difficult to conceive what sort of stimulus could cause reversals in both directions.

We are currently unable unequivocally to identify an environmental stimulus which might have triggered the formation of the *frondosa* leaves we observed in 1984 at Mountain Lake. A weather station is located on the Biological Station grounds only a few meters from some of the plants in question. Records from this station over several years show no highly unusual conditions, although a cold snap *did* occur in late May of 1984 with low temperatures recorded at 31°F (−1°C). We would like to suggest the possibility that grazing by deer may have been a cause. Substantial grazing on *O. cinnamomea* was observed in the Mountain Lake area in 1982 (W. H. Wagner, pers. comm.). It is possible that instances of severe grazing during 1982 or 1983 may have resulted in the highly localized pattern of *frondosa* occurrence we observed in 1984.

The possibility that injury is a cause of this interesting phenomemon suggests that it may be an adaptive response. A reduction in the amount of photosynthetic area caused by injury could be partially compensated by conversion of presumptive sporogenous tissue into photosynthetic tissue. It would be interesting to attempt to induce this response experimentally by removing leaves from *O. cinnamomea* plants at various times during the growing season.

We are grateful for facilities and weather records provided by the Mountain Lake Biological Station and would like to thank James Caponetti for helpful comments on the manuscript and Margaret Wren for providing details of her findings in South Carolina.

Literature Cited

Beitel, J. M., W. H. Wagner, Jr., and K. S. Walter. 1981. Unusual frond development in sensitive fern *Onoclea sensibilis* L. Amer. Midl. Naturalist 105:396–400.

Britton, N. L. 1890. *Catalogue of plants found in New Jersey.*

Harvey, W. H. and J. D. Caponetti. 1972. *In vitro* studies on the induction of sporogenous tissue on leaves of cinnamon fern. I. Environmental factors. Canad. J. Bot. 50:2673–2682.

——— and ———. 1973. *In vitro* studies on the induction of sporogenous tissue on leaves of cinnamon fern. II. Some aspects of carbohydrate metabolism. Canad. J. Bot. 51:341–349.

——— and ———. 1974. *In vitro* studies on the induction of sporogenous tissue on leaves of cinnamon fern. III. The role of reduced nitrogen. Canad. J. Bot. 52:2611–2614.

House, H. D. 1933. Additions to the fern flora of New York state. Amer. Fern J. 23:1–7.

Kittredge, E. M. 1941. An *Osmunda cinnamomea* variant. Amer. Fern J. 31:71–72.

McLouth, C. D. 1897. Abnormal fruiting forms of *Osmunda cinnamomea.* Fern Bull. 5:7–8.

Owen, M. L. 1901. Ferns of Mt. Toby, Massachusetts. Rhodora 3:41–43.

Steeves, T. A. 1959. An interpretation of two forms of *Osmunda cinnamomea.* Rhodora 61:223–230.

——— and W. R. Briggs. 1958. Morphogenetic studies on *Osmunda cinnamomea* L.: The origin and early development of vegetative fronds. Phytomorphology 8:60–72.

——— and R. H. Wetmore. 1953. Morphogenetic studies on *Osmunda cinnamomea* L.: Some aspects of the general morphology. Phytomorphology 3:339–354.

Sussex, I. M. and T. A. Steeves. 1958. Experiments on the control of fertility of fern leaves in sterile culture. Bot. Gaz. (Crawfordsville) 119:203–208.

Torrey, J. 1840. Report on the Botanical Department of the Geological Survey of New York. Assembly, No. 50:113–197.

Shorter Notes

Gametophytes of *Equisetum giganteum*.—In a recent article, Duckett and Pang (J. Linn. Soc., Bot. 88:11–34, 1984) claimed that sexual behavior of *Equisetum giganteum* L. was similar to that of the other species of *Equisetum* subg. *Hippochaete*, with homosporous spores producing either antheridial or archegonial gametophytes. The archegonial gametophytes as they age begin to produce antheridial lobes and eventually cease archegonial production. The antheridial gametophytes remain so, or with age and by lamellar proliferation also produce archegonial branches. I had earlier described *E. giganteum* gametophytes as different from those of any other species because they were normally bisexual, that is, simultaneously hermaphroditic, with both gametangia being borne upon the same meristem, rather than having separate antheridial lobes as the sequentially hermaphroditic species do (Hauke, Beih. Nova Hedwigia 8:1–123, 1963; Bull. Torrey Bot. Club 96:568–577, 1969). On this basis I had classified *E. giganteum* as a distinct section *Incunabula*, and theorized on evolution of heterothallism in *Equisetum*.

Duckett and Pang speculated that my culturing procedure, using mass cultures, resulted in a very rapid shift from female to male, which I then interpreted as simultaneous hermaphroditism, rather than rapid sequential hermaphroditism. They cited a plant in cultivation in the Botanic Gardens, Edinburgh as the source of the spores from which they grew their *Equisetum* gametophytes. I wrote to the Botanic Gardens at Edinburgh requesting a specimen from the plant in question. Dr. C. N. Page was gracious enough to send me a fine specimen of that plant, and noted that it was originally collected in Mexico. Although it is superficially much like *E. giganteum*, the Edinburgh plant is *E. myriochaetum* Schlecht. and Cham. That species has stomata in a single line on each side of the internodal grooves, rather than rows 2 to 4 stomata wide. The branch ridges viewed in profile show a sawtooth pattern rather than a blocky pattern. The stem sheath segments are flattened and green, rather than rounded and light brown. So far as I have seen, all large branched *Equisetum* collections from Mexico are either *E. myriochaetum* or the hybrid *E.* ×*schaffneri*, and *E. giganteum* does not occur in that country.

As *E. myriochaetum* rather than *E. giganteum*, the gametophytes described by Duckett and Pang are in agreement with my (1969) descriptions of that species, and their challenge to my classifications of *Equisetum* subg. *Hippochaete* and my hypothesis of evolution of heterothallism in the genus *Equisetum* is not supported.—Richard L. Hauke Department of Botany, University of Rhode Island, Kingston, RI 02881.

***Marsilea macropoda* New to Louisiana**—During February, 1982, one of us (SH) discovered a population of *Marsilea* growing within the city limits of New Orleans. We identified the plants as *Marsilea macropoda* Engelm. ex A. Braun. The population occurs in an old abandoned lot. The situation is suggestive of persistence after cultivation; however, local residents could not substantiate this. The lot is littered with gravel and concrete rubble, indicating that the plants might also have been introduced through disturbance and may have persisted at the locality. The population is large and vigorous, and at the time of collection most plants were fertile. Specimens from the population (*Landry 7895*) are deposited at LAF, SMU, US, and NY. *Marsilea macropoda* is readily distinguishable from other species known to occur in Louisiana, *M. uncinata* and *M. vestita*, by its markedly more robust stature, the lack of a prominent tooth on the sporocarps, and the frequent presence of two or more sporocarps per peduncle. *Marsilea macropoda* is otherwise known from south-central Texas, where it previously has been regarded as a Texas endemic (Correll & Johnston, *Manual of the Vascular Plants of Texas*, Texas Research Foundation, Renner, TX, 1970). The Louisiana population represents a range extension of approximately 400 miles for the species.—GARRIE P. LANDRY, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504; and SAMUEL W. HOLDER, Jefferson Parish Environmental and Development Control Department, 3600 Jefferson Hwy., Jefferson, LA 70121.

E. A. C. L. E. Schelpe (1924–1985)

Professor Edmund André Charles Lois Eloi ('Ted') Schelpe died after a sudden heart attack at his home on 12 October 1985. He was born in Durban, South Africa, on 27 July 1924 and completed a BSc with distinction in Botany at Natal University in 1943; at the same University he obtained an MSc (Class I) in 1946. In 1952, he was awarded the DPhil degree of Oxford University (Wadham College). He held the following posts at the University of Cape Town: Lecturer in Botany, 1953–1954; Senior Lecturer and Curator of the Bolus Herbarium, 1954–1958; Associate Professor and Curator of the Bolus Herbarium, 1968–1973, the title Curator being changed to Director in 1970; Professor (ad hominem) and Director of the Bolus Herbarium, 1973–1985. Professor Schelpe was an elected Fellow of the Royal Society of South Africa, the Linnean Society of London, and the University of Cape Town. From 1964 he was a member of the Committee on Pteridophyta of the International Association of Plant Taxonomists. Among voluntary societies, he played a valuable role in helping found the Orchid Society of Southern Africa and was President of the Botanical Society of Southern Africa.

He made major contributions to the systematics of Pteridophyta in southern Africa. A few days before he passed away, he finished checking the final proofs of the volume on Pteridophyta for the *Flora of Southern Africa*. This was a culmination of studies during the course of which he published accounts of the Pteridophyta for the *Flora Zambesiaca*, the *Flora de Moçambique*, and the Con-

spectus *Florae Angolensis*. He published over 100 scientific papers and books, which besides Pteridophyta covered topics in orchids, bryophytes, plant ecology, and phytogeography. A complete bibliography of Professor Schelpe's publications will appear in Bothalia, volume 16, part 2.—From material provided through the kindness of A. V. HALL, University of Cape Town, South Africa.

1986 AIBS MEETING—CALL FOR PAPERS

The American Fern Society and the Botanical Society of America will meet with the AIBS Annual Meeting at the University of Massachusetts, Amherst, on 10–14 August 1986. Those members of the American Fern Society wishing to present a paper or poster and who have not received abstract forms may obtain forms from the program chairman of the American Fern Society: DR. FLORENCE S. WAGNER, Department of Botany, University of Michigan, Ann Arbor, MI 48109.

Referees, 1985—I thank the Associate Editors and referees listed below for their valuable assistance in the review process. Their evaluations of manuscripts submitted to American Fern Journal have aided authors, made my job easier, and contributed to the quality of our journal.—ALAN R. SMITH

David S. Barrington
Brent Berlin
David E. Bilderback
Donald M. Britton
Ralph E. Brooks
Raymond B. Cranfill
Jeffrey G. Duckett
Ernest M. Gifford, Jr.
Richard L. Hauke
R. James Hickey
David M. Johnson
Donald R. Kaplan
Jon E. Keeley
Terry W. Lucansky
Michael R. Mesler
John T. Mickel
John H. Miller
James D. Montgomery
Richard Mueller
Clifton E. Nauman
James H. Peck
Michael G. Price
V. Raghavan
P. Mick Richardson
Rudolf Schmid
David Seigler
Darlene Southworth
Robert G. Stolze
W. Carl Taylor
Alice Tryon
Rolla Tryon
David H. Wagner
Warren H. Wagner, Jr.

Index to Volume 75—1985

Classified entries: botanical names (new names in **boldface**); major subject headings, including key words from titles; reviews (grouped, listed by last name of first author of work reviewed). Names of authors, followed by titles of articles or references to first authors, are listed alphabetically in Table of Contents, iii–iv.

AIBS meeting, 1986, call for papers, 134
Alsophila imrayana var. **basilaris**, 31
Anatomy: Cnemidaria, 80; Cyatheaceae, 80; Equisetum 111; Sphaeropteris, 80
Anemia: subg. Coptophyllum, 36, 37; **A. antrorsa**, 33; **A. ayacuchensis**, 36; A. elegans, 37; A. jaliscana, 36; **A. porrecta**, 36; A. tomentosa var. australis, 33

Asplenium: A. subnormale, 73; A. unilaterale, 73
Atomic composition, of Onoclea spores, 1
Azolla: A. mexicana, 38; sporopollenin content of spore apparatus, 38
Blechnum occidentale, 1
Bolbitis heudelotii, 73
Cnemidaria: C. mutica, 81; anatomy, 80
Ctenopteris, 6; C. brevivenosa, 9; C. fabaespora, 9; C. lanigera, 8; C. longa, 9; C. senilis, 9; C. sericeolanata, 9; C. stella, 8
Culcita coniifolia, 13
Cyatheaceae: Cnemidaria, 80; Sphaeropteris, 80; Trichipteris, 92
Cyrtomium, 23
Dicranopteris flexuosa, 79
Dryopteris: D. ×australis, 71; D. goldiana, 23; D. ludoviciana, 71
Ecology: aquatic ecotype of Asplenium unilaterale, 73; branching patterns in Lophosoria, 105; colonization of Volcan Chichonal by Pityrogramma, 1
Equisetum: E. arvense, 112; E. diffusum, 112; E. hyemale, 112; E. telmateia, 112; gametophytes of E. giganteum, 132; E. sylvaticum, 120; ontogeny of commissure, 111
Ethnopteridology, of Chácobo Indians, 19
Evolution: Polystichum, 22
Gametophytes: Dicranopteris, 79; Equisetum giganteum, 132; E. sylvaticum, 120
Glyphotaenium trifurcatum, 31
Grammitidaceae, 6
Grammitis, 6; G. cultrata, 8; G. lanigera, 8; G. senilis, 9
Hybridization, in Polystichum, 22
Indians, Chácobo, 19
Isoëtes: I. acadiensis, 44; I. eatonii, 44; I. echinospora, 44; I. engelmannii, 44; I. hieroglyphica, 44; I. macrospora, 44; I. melanopoda, 77; I. riparia, 44; I. tegetiformans, 102; I. tuckermanii, 44; morphology, 44
Loxogramme, 9
Lophosoria quadripinnata, branching pattern, 105
Lycopodium, 39
Marsilea: M. crenata, 30; M. macropoda, 133; M. mucronata, 28; M. mollis, 30; M. oligospora, 30; M. quadrifolia, 28; M. vestita, 28; longevity of sporocarps, 30
Morphology: Equisetum, commissure, 111; Isoëtes, leaves and sporangial region, 44; Trichipteris, spores, 92
Obituary, E. A. C. L. E. Schelpe (1924–1985), 133
Onoclea sensibilis, atomic composition of spores, 12
Osmunda cinnamomea f. frondosa, 128
Papuapteris, 23
Pecluma, 6
Phanerophlebia, 23
Pityrogramma calomelanos, 1
Plecosorus, 23
Polypodiaceae, 6
Polypodium, 6; P. alternifolium, 9; P. longum, 9; P. pectinatum-plumula complex
Polystichum: Symposium, 22; taxonomy, 22; sect. Metapolystichum, 25; P. aleuticum, 72; P. acrostichoides, 23, 25; P. braunii, 24; P. dictyophyllum, 26; P. dubium, 26; P. echinatum, 25; P. fournieri, 25; P. imbricans, 24; P. kruckebergii, 24; P. lehmannii, 25; P. lemmonii, 24; P. lonchitis, 23; P. mickelii, 25; P. munchii, 25; P. muricatum, 24; P. munitum, 24; P. platyphyllum, 24, 25; P. polyphyllum, 25; P. scopulinum, 24; P. setigerum, 24; P. speciosissimum, 25
Prosaptia contigua, 9
Psilotum nudum, 13
Pteridium aquilinum, 109
Range extensions/deletions: Alaska, Adak Island, Polystichum aleuticum, 72; Arkansas, Dryopteris ludoviciana, D. ×australis, 71; Florida, Dicranopteris flexuosa, 79; Georgia, Isoëtes tegetiformans, 102; Kansas and Missouri, Marsilea quadrifolia, M. vestita, 28; Louisiana, Isoëtes melanopoda, 77; Marsilea macropoda, 133
Referees, 1985, 134
Reviews: Foster, F. Gordon, Ferns to know and grow, 11; Jacobsen, W. B. G., The ferns and fern allies of Southern Africa, 31; Jarrett, F. M., et al., Index filicum/Supplementum quintum: pro annis 1961–1975, 76; Pant, D. D., Gametophytes of Ophioglossaceae, 18; Prelli, Remy, Guide de fougeres et plantes alliees, 104; Proctor, George R., Ferns of Jamaica, a guide to the pteridophytes, 119; Taylor, W. Carl, Arkansas ferns and fern allies, 37
Schelpe, E. A. C. L. E. (1924–1985), obituary, 133
Selaginella: kraussiana, 13, 40; aff. schizobasis, 1
Sphaeropteris: S. elongata, 81; anatomy, 80
Spores: Anemia, 35; Azolla, 38; Grammitis, 6; Lycopodium, 39; Onoclea, 12; Polypodium, 6; Trichipteris, 92
Sporopollenin, of Azolla, 38
Symposium, Polystichum, 22
Thelypteris: subg. Amauropelta, 56; subg. Goniopteris, 63; **T. abdita**, 63; **T. cordata** var. **imitata**, 63; **T. frigida**, 31; **T. hastata** var. **heterodoxa**, 69; **T. hildae**, 67; **T. inabonensis**, 61; **T. namaphila**, 56; **T. rheophyta**, 58; **T. verecunda**, 65
Trichipteris corcovadensis, 92
Volcán Chichonal, 1

U.S. Postal Service

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

Required by 39 U.S.C. 3685)

1A. TITLE OF PUBLICATION	1B. PUBLICATION NO.	2. DATE OF FILING
AMERICAN FERN JOURNAL	0 0 0 2 8 4 4 4	16 Oct 1985

3. FREQUENCY OF ISSUE	3A. NO. OF ISSUES PUBLISHED ANNUALLY	3B. ANNUAL SUBSCRIPTION PRICE
quarterly	4	$8 mem; $9 subscr

4. COMPLETE MAILING ADDRESS OF KNOWN OFFICE OF PUBLICATION *(Street, City, County, State and ZIP+4 Code) (Not printers)*

Pringle Herbarium, University of Vermont, Burlington, VT 05405

5. COMPLETE MAILING ADDRESS OF THE HEADQUARTERS OF GENERAL BUSINESS OFFICES OF THE PUBLISHER *(Not printer)*

Pringle Herbarium, University of Vermont, Burlington, VT 05405

6. FULL NAMES AND COMPLETE MAILING ADDRESS OF PUBLISHER, EDITOR, AND MANAGING EDITOR *(This item MUST NOT be blank)*

PUBLISHER *(Name and Complete Mailing Address)*

AMERICAN FERN SOCIETY, INC. (see 5.)

EDITOR *(Name and Complete Mailing Address)*

Dr. Alan R. Smith, Dept. of Botany, Univ. of California, Berkeley, CA 94720

MANAGING EDITOR *(Name and Complete Mailing Address)*

none

7. OWNER *(If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Item must be completed.)*

FULL NAME	COMPLETE MAILING ADDRESS
AMERICAN FERN SOCIETY, INC.	(see 5.)

8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES *(If there are none, so state)*

FULL NAME	COMPLETE MAILING ADDRESS
none	

9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES *(Section 423.12 DMM only)*
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes *(Check one)*

[X] (1) HAS NOT CHANGED DURING PRECEDING 12 MONTHS

[ ] (2) HAS CHANGED DURING PRECEDING 12 MONTHS *(If changed, publisher must submit explanation of change with this statement.)*

10. EXTENT AND NATURE OF CIRCULATION *(See instructions on reverse side)*	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES *(Net Press Run)*	1400	1400
B. PAID AND/OR REQUESTED CIRCULATION 1. Sales through dealers and carriers, street vendors and counter sales	0	0
2. Mail Subscription *(Paid and/or requested)*	1204	1096
C. TOTAL PAID AND/OR REQUESTED CIRCULATION *(Sum of 10B1 and 10B2)*	1204	1096
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	1	3
E. TOTAL DISTRIBUTION *(Sum of C and D)*	1205	1099
F. COPIES NOT DISTRIBUTED 1. Office use, left over, unaccounted, spoiled after printing	195	301
2. Return from News Agents	0	0
G. TOTAL *(Sum of E, F1 and 2—should equal net press run shown in A)*	1400	1400

11. I certify that the statements made by me above are correct and complete	SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER
	Alan R. Smith Alan R. Smith, editor

PS Form 3526, July 1984

(See instruction on reverse)

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

AMERICAN FERN JOURNAL

Volume 76

Number 1

January–March 1986

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Isoëtes Megaspore Surface Morphology: Nomenclature, Variation, and Systematic Importance — *R. James Hickey* — 1

Active Enzymes from Megaspores of *Marsilea* and *Regnellidium* — *Douglas E. Soltis* and *Pamela S. Soltis* — 17

Germinating Spores and Growing Sporelings of Aquatic *Isoëtes* — *W. Carl Taylor* and *Neil T. Luebke* — 21

Shorter Notes

***Pellaea brachyptera* New to Washington** — *Edward R. Alverson* and *Joseph Arnett* — 25

A Second Eastern North American Occurrence for Forked Spleenwort, *Asplenium septentrionale* — *Eleanor M. Bush* — 26

Second Locality for *Dryopteris carthusiana* in Arkansas — *James H. Peck* — 28

Reviews — 29, 30

Information for Authors — Cover 3

[redacted] BOT

JUN 1

The American Fern Society

Council for 1986

TERRY R. WEBSTER, Biological Sciences Group, University of Connecticut, Storrs, CT 06268. *President*

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH ..... Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER ..... Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER ..... U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER ... Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 76(1):1–16 (1986)

Isoëtes Megaspore Surface Morphology: Nomenclature, Variation, and Systematic Importance

R. James Hickey
Botany Department, Miami University, Oxford, OH 45056

The Isoëtaceae is a small, cosmopolitan family of heterosporous pteridophytes within the lycopsid line of evolution. Based on a number of shared derived characters, such as a ligule and heterospory, it appears to be most closely related to the extant family Selaginellaceae. Unlike the latter, members of the Isoëtaceae are aquatic or are associated with seasonally inundated habitats. The family is monotypic: the genus *Isoëtes* comprises approximately 150 species (Tryon & Tryon, 1982). The exact number of species is difficult to establish because of discrepancies among authorities regarding species delimitation and because *Isoëtes* in many parts of the world is still poorly known.

Systematic studies in the genus have been greatly hampered by both the lack of collections and the tremendous degree of phenotypic plasticity; as stated by Kott (1980) "perhaps the only constant in this group is its variation." Although a thorough study of the genus leaves no doubt as to the validity of most species (Boom, 1982; Kott & Britton, 1983; Hickey, 1985), identification of taxa is difficult because of the paucity and variability of characters. As a result of these problems, and the small number of collections per species, workers have placed special emphasis on one or two characters and have erected infrageneric classification systems around them. The first such classification was proposed by Braun (fide Fuchs, 1962) and was based on habitat preference, primarily as inferred from leaf morphology. In general this classification system was widely accepted; subsequent treatments (e.g. Baker, 1880; Motelay & Vendryes, 1882; Eaton, 1900) included some hierarchical changes, but the underlying theme remained unchanged. As a result of better specimen quality and more exact field data, it soon became apparent that this classification system was both unnatural and simplistic (Engelmann, 1882; Eaton, 1900). West and Takeda (1915) made the first break with implied habitat characteristics by stressing anatomical differences. While their emphasis on primary, observable characters is noteworthy, it should be realized that their characters are essentially the same ones that Braun (1864) used to distinguish between terrestrial and aquatic habitats.

In 1922, Pfeiffer classified members of the family according to megaspore surface ornamentation (Table 1). Her reason for rejecting previous classifications is not directly stated but seems to stem from the unreliability of habitat data and from her understanding of evolutionary changes in megaspore surface morphology. Pfeiffer recognized four basic megaspore surface patterns and, based on these, established sections *Tuberculatae*, *Echinatae*, *Cristatae*, and *Reticulatae*. Despite criticisms regarding the reliability of megaspore morphology (Engelmann, 1882; Eaton, 1900; Pfeiffer, 1922; Duthie, 1929), and the lack of corre-

TABLE 1. Infrageneric Classification System in *Isoëtes* by Pfeiffer (1922), as Modified by Fuchs (Fuchs 1962; Fuchs-Eckert 1981).

Section	Spore type
Isoëtes (=*Cristatae* Pfeiffer)	cristate
Terrestres A. Br. (=*Reticulatae* Pfeiffer)	reticulate
Palustres A. Br. (=*Tuberculatae* Pfeiffer)	tuberculate
Echinatae Pfeiffer	echinate
Laevis Fuchs	levigate

spondence between megaspore morphology and other characters (Berthet & Lecocq, 1977), her classification system has been almost universally accepted. In 1981, Fuchs-Eckert added a fifth section, *Laevis*, which is characterized by smooth megaspores.

During revisionary studies on Neotropical species (Hickey, 1985), it became obvious that there were additional problems associated with this reliance on megaspore surface morphology. In particular, use of this single character set has obscured phylogenetic relationships (Hickey, 1985, 1986a). The problems of this one-character taxonomy are amplified by overly simplistic and non-discriminatory megaspore character state assessments and by a failure to look for correlations with other characters (and its corollary, reasons for a lack of such correlations). Multiple character analyses are necessary to determine the limits and affinities of *Isoëtes* species (Hickey, 1985, 1986a, b). Within such analyses, megaspore morphology remains one of the most significant sources of phylogenetically informative characters. It is essential, however, that the particular states be accurately and completely described. The primary purpose of this paper is to present a more precise, expanded nomenclature for the character states of *Isoëtes* megaspore ornamentation. In addition, the related problems of character variation, homoplasy, and the systematic importance of megaspore data are discussed. While all of these facets are interconnected, it is simpler to discuss terminology, spore maturity, polymorphism, and convergence separately.

TERMINOLOGY

Surface morphology.—While Pfeiffer (1922) recognized only four formal categories of megaspore ornamentation, her discussions suggest that she was aware of a greater diversity of forms. For example, within sect. *Tuberculatae* she recognized warty, tuberculate, and papillate types as well as spores in which the ornamentation "fail(s) to appear, leaving a smooth surface." Because of the difficulty in describing this diversity she relied largely on photographs of the spores; unfortunately these photographs were poorly reproduced and therefore of relatively little use. Even with the addition of a fifth spore type (smooth or levigate, Fuchs-Eckert, 1981) the diversity of surface morphology far exceeded the number of recognized types. Although the use of only five spore types simplified the identification of ornamentation patterns, the inclusiveness of the terminology would not allow for discrimination between otherwise similar taxa. As an alter-

TABLE 2. Types of Megaspore Surface Morphology in *Isoëtes*: Descriptions, Previous Classification, and Specific Examples.

Tuberculate:	Spores with moderately broad, radially symmetrical projections, as high as wide, with straight, inclined sides and an acute apex. Examples—*I. bradei, I. luetzelburgii, I. melanopoda.* (Figs. 1a, b, 4a)
Pustulate:	Spores with broad, radially symmetrical projections, as wide as high or wider, with convex sides and an obtuse, rounded apex. Previously classified as tuberculate. Examples—*I. cubana, I. triangula.* (Figs. 1c, d)
Levigate:	Spores without apparent ornamentation, smooth. Previously classified as tuberculate or levigate. Examples—*I. lechleri, I. mexicana, I. gunnii.* (Figs. 1e, f)
Saccate:	Spores with radially to bilaterally symmetrical, bulbous projections which appear deflated and collapsed. Previously classified as tuberculate. Example—*I. gigantea.* (Fig. 2a)
Clavate:	Spores with radially symmetrical projections which are approximately as high as wide, with an obtuse, expanded apex. Previously classified as tuberculate. Examples—*I. ovata*, a subclavate condition is seen in *I. clavata.* (Fig. 2b)
Verrucate:	Spores with radially symmetrical projections, as high as or slightly higher than wide, with straight parallel sides and an obtuse apex. Previously classified as tuberculate. Example—*I. gardneriana.* (Fig. 2c)
Baculate:	Spores with narrow, radially symmetrical projections 1½ or more times as high as wide, with straight, parallel sides and an obtuse, rounded apex. Previously classified as tuberculate. Examples—*I. panamensis, I. baculata.* (Figs. 2d–f)
Echinate:	Spores with narrow, radially symmetrical (or nearly so) projections, at least 1½ times as high as wide, with straight, inclined sides and an attenuate, pointed apex. Previously classified as echinate. Examples—*I. echinospora*, some specimens of *I. storkii* and *I. andina.* (Figs. 5d, e)
Rugulate:	Spores with broad, distinct muri, as wide as or wider than tall, with a broadly rounded crest; the muri not forming distinct areoles. Previously classified as cristate. Examples—*I. savatieri, I. herzogii.* (Figs. 3a–c, 5b, c)
Cristate:	Spores with thin, distinct muri, taller than broad, usually with an apically serrate crest; not forming distinct areoles. Previously classified as cristate. Examples—*I. storkii, I. ulei, I. andina.* (Figs. 3d, 5f)
Reticulate:	Spores with thin, distinct muri, taller than broad, usually with an apically serrate crest; forming distinct areoles. Previously classified as reticulate. Examples—*I. engelmannii, I. martii, I. novo-granadensis.* (Figs. 3e, f)
Retate:	Spores with broad, distinct muri, as wide as or wider than tall, with a broadly rounded crest; forming distinct areoles. Previously classified as reticulate. Examples—*I. durieui, I. stevensii.* (See fig. Va in Berthet & Lecocq, 1977, and fig. 7 in Croft, 1980)

native, I propose a more discriminatory megaspore terminology circumscribing twelve defined morphological types (Table 2). While the inherent variability and intergradation in surface morphology precludes an all-encompassing classification, the additional seven categories are distinct enough to merit recognition, and will allow for more rapid and accurate identification of taxa. Definitions (Table 2) and scanning electron micrographs (Figs. 1–5) are included to provide a basis for standardization and stability of nomenclature.

Spore types were initially characterized from Neotropical specimens and the resultant states compared with spores of extra-Neotropical species. A subsequent literature survey and an examination of *Isoëtes* specimens from A, GH, and NY revealed only one additional ornamentation type. This retate spore pattern is similar in morphology to the more common reticulate spore type. However, the

two types differ in their evolutionary origins: retate spores are the result of anastomosing rugae or tubercles while reticulate spores are the result of anastomosing cristae. Retate spores are found in *I. durieui* Bory (fig. Va in Berthet & Lecocq, 1977), *I. stevensii* Croft (fig. 7 in Croft, 1980), and several other Old World species.

Even with the recognition of an increased number of megaspore types, some spores are intermediate in morphology and do not fit neatly into one of the twelve categories. Such spore patterns are best described by means of hyphenated terms, e.g. *echinate-cristate* or *cristate-reticulate*.

Secondary ornamentation.—In addition to gross surface morphology, other aspects of the spore surface have systematic application. One such character is the change in density and discreteness of ornamentation on the distal spore surface immediately adjacent to the equatorial ridge. Changes in this region may take the form of a decrease (Figs. 1b, 5c), an increase (Figs. 3d–f, 5e, f), or the total loss of ornamentation (Figs. 4e, 5a, b). This region of differential ornamentation, the girdle, is useful in the identification of *I. acadiensis*, *I. hieroglyphica* A. A. Eaton, *I. macrospora* (Kott & Britton, 1983), *I. weberi* Herter, *I. montezumae* A. A. Eaton, *I. savatieri* Franchet, and *I. storkii* Palmer (Hickey, 1985).

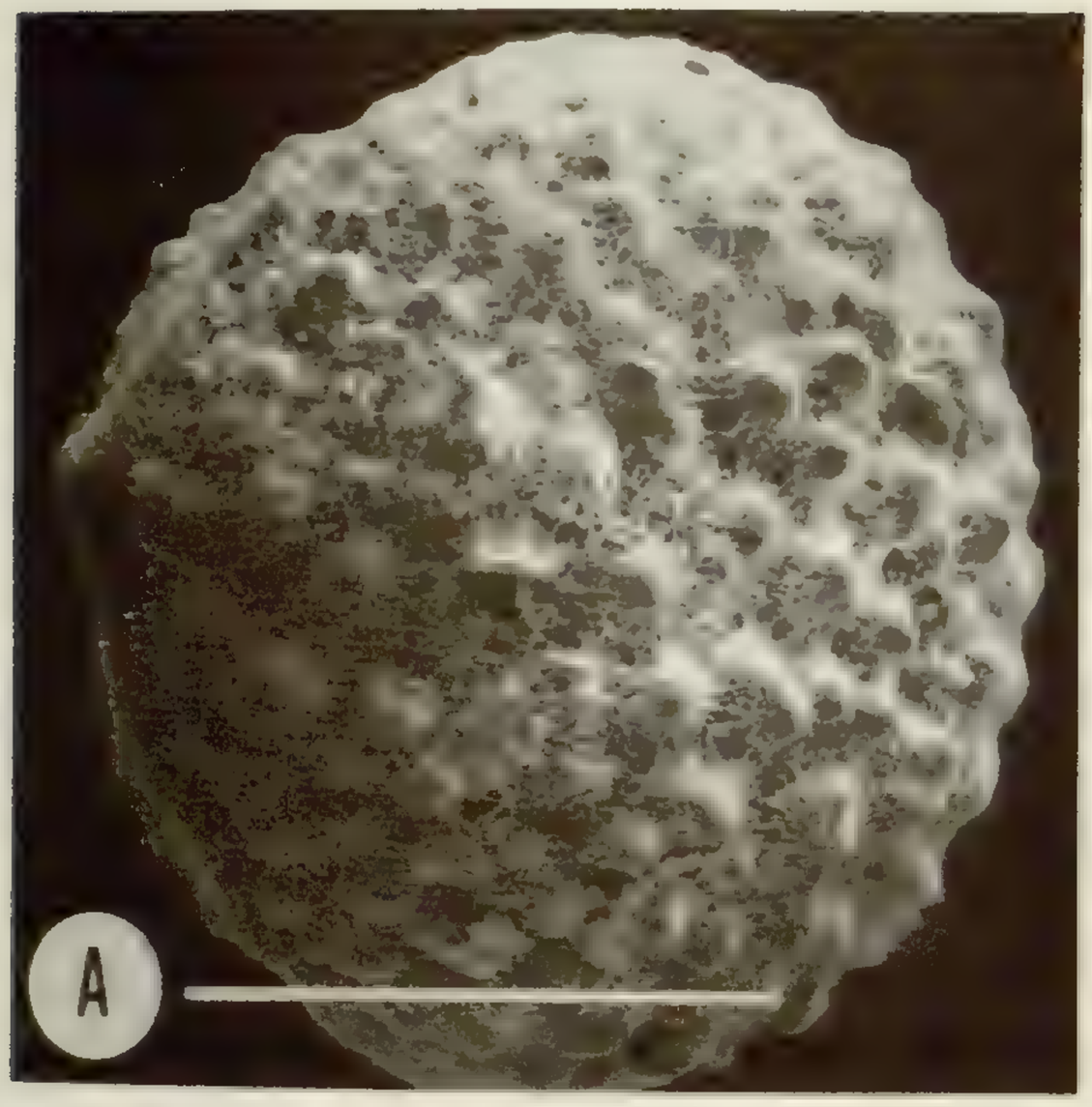
A second suite of characters, which is associated with the variation in form of the equatorial and proximal ridges, has rarely been used as a source of systematic characters. However, a number of tropical species do possess stable and unusual ridge features that are of systematic value (Hickey, 1985). For example, *I. gigantea* U. Weber is readily distinguished from all other Neotropical species by its extremely narrow, knife-like proximal ridges (Fig. 2a). In morphologically similar species pairs such as *I. cubana* Baker and *I. triangula* Weber, the cross-sectional shapes of the equatorial and proximal ridges (i.e. whether rounded or triangular) are diagnostic (Hickey, 1985). *Isoëtes gardneriana* Mett. and, to a lesser extent, *I. panamensis* Maxon & Morton are characterized by an unusual extension of the equatorial ridge that projects outwardly at the junctures with the proximal ridges (Figs. 2c, d). This distinctive flange is also found in *I. melanotheca* Alston

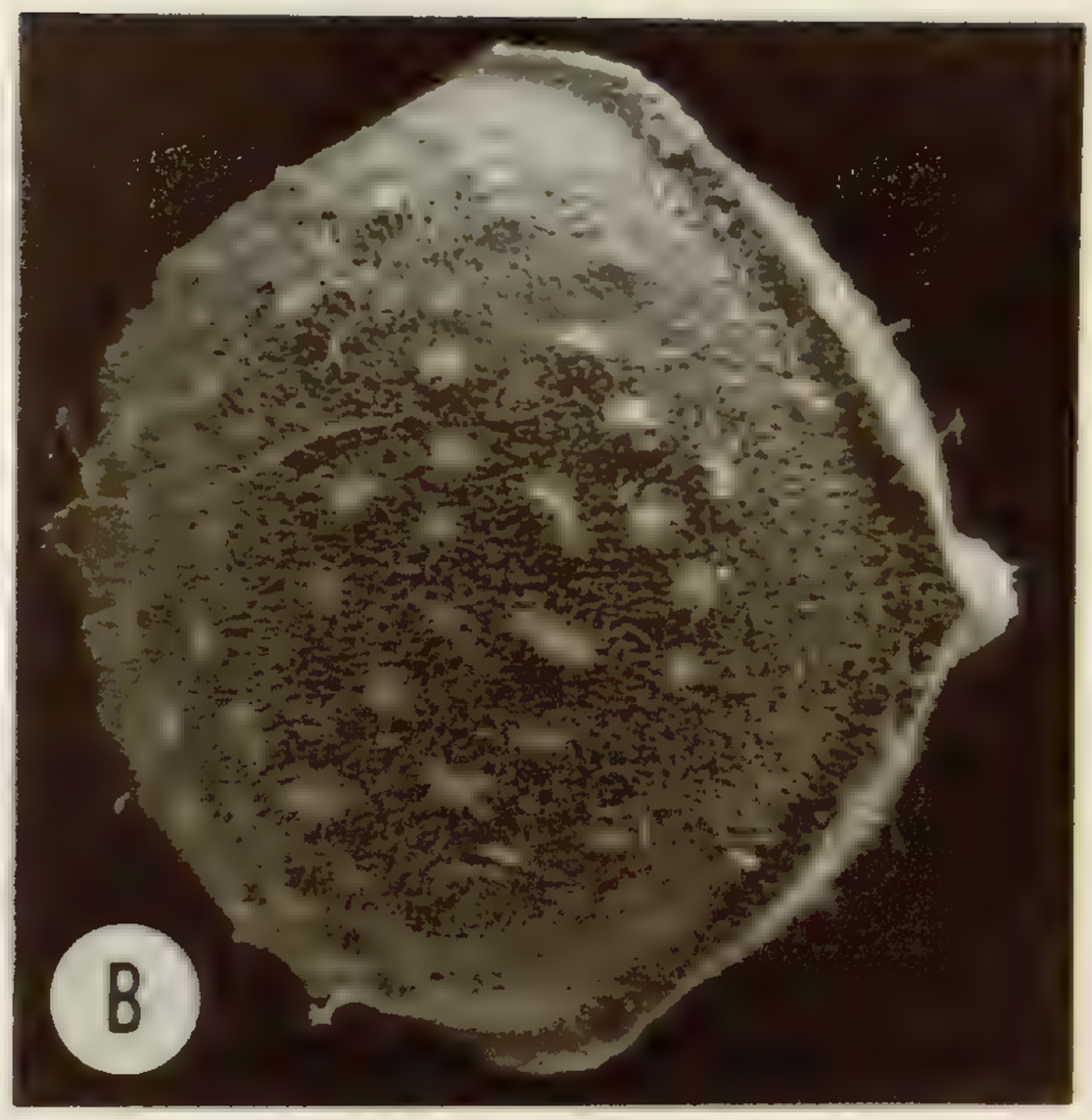
→

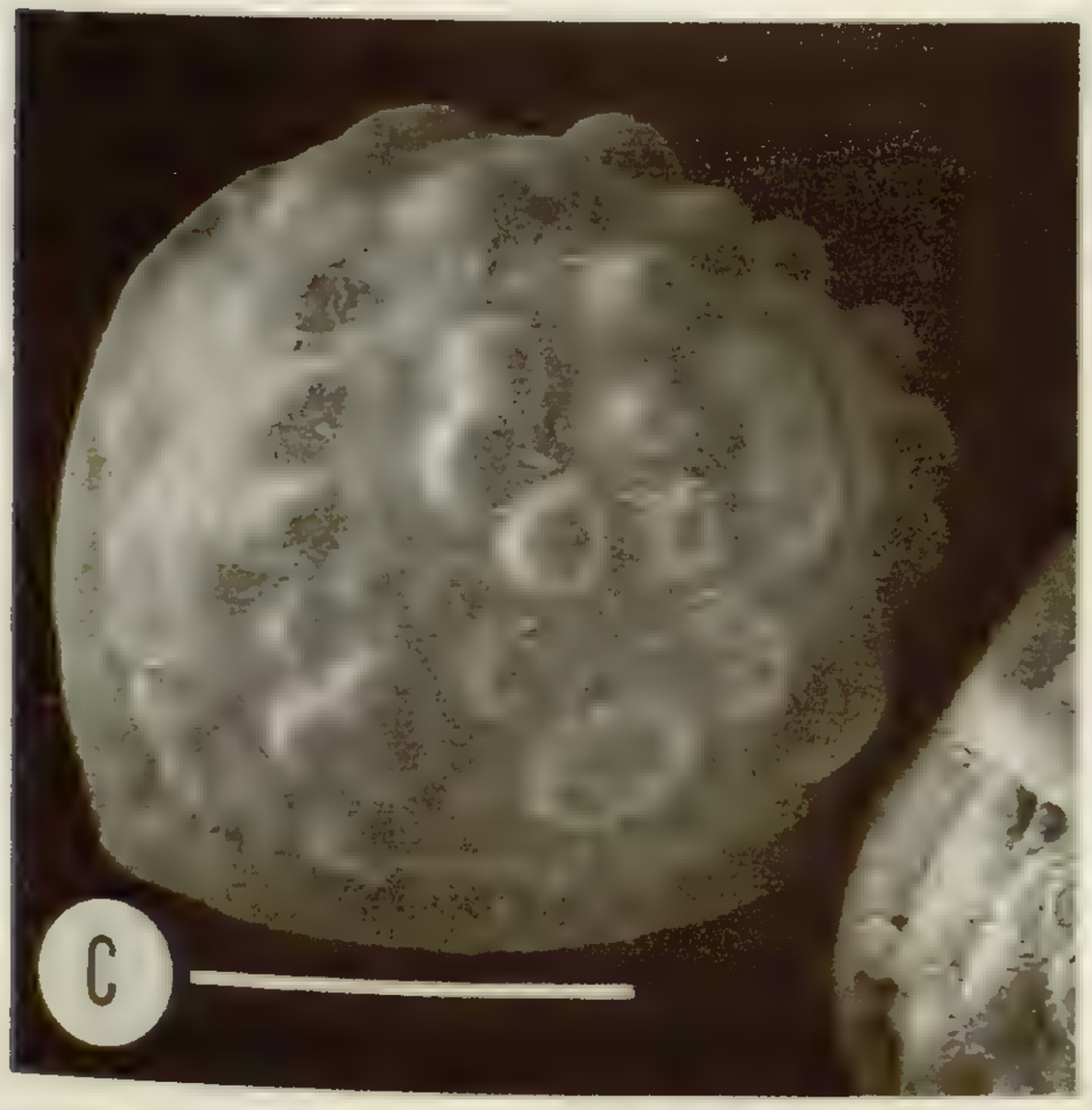
FIG. 1. Megaspore surface morphology in *Isoëtes*. Tuberculate form: a, *I. luetzelburgii*, *Luetzelburg 13709* (M); b, *I. weberi*, *Herter 2273* (MO). Pustulate form: c, *I. cubana*, *Wright s.n.* (MO); d, *I. triangula*, *Kral 25320* (MICH). Levigate form: e, *I. glacialis*, *Hickey 753 & Eshbaugh* (MU); f, *I. mexicana*, *McVaugh 21725* (NY). Bar = 200 μm.

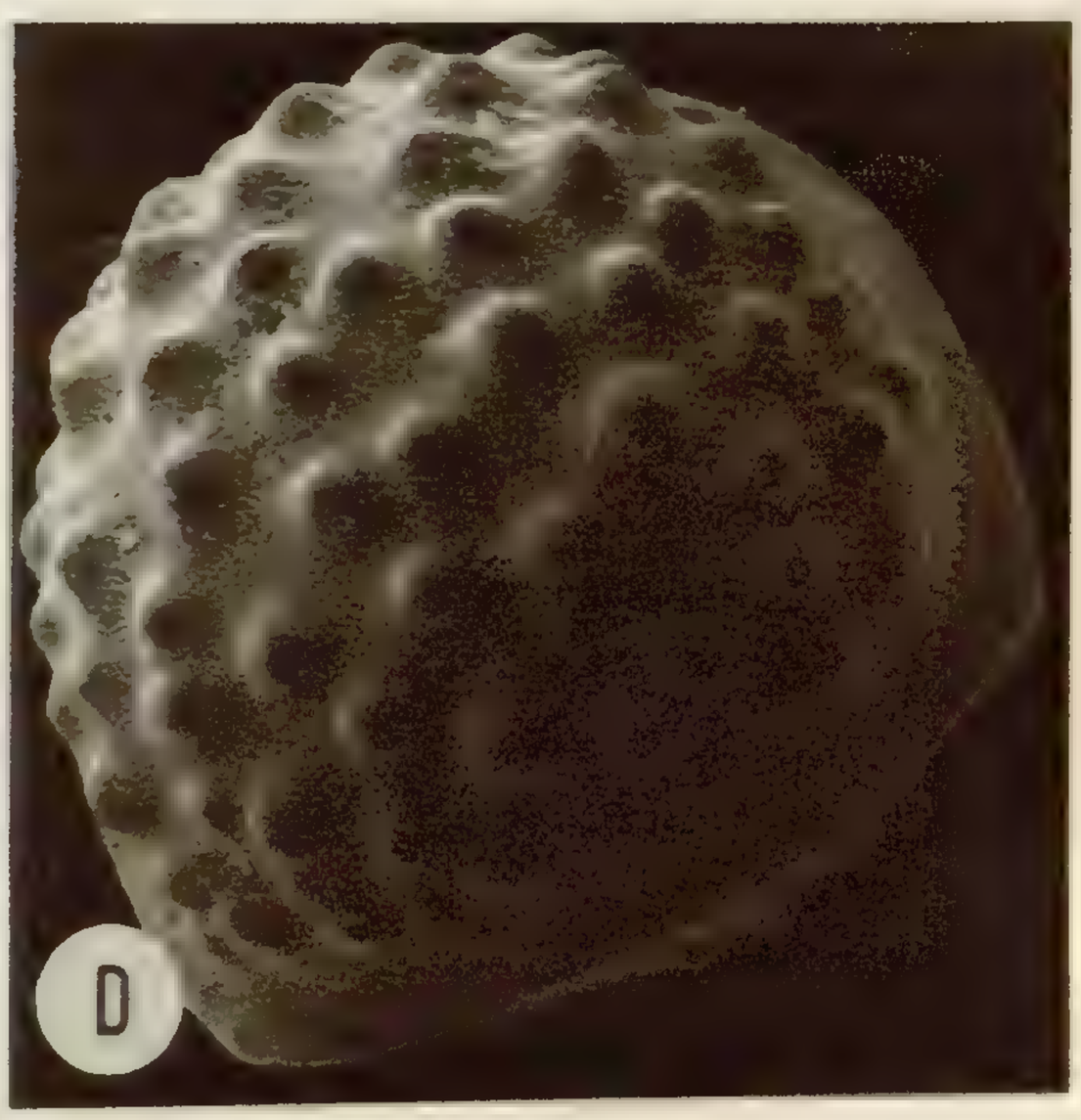
FIG. 2. Megaspore surface morphology in *Isoëtes*. Saccate form: a, *I. gigantea*, *Luetzelburg 15057* (M). Subclavate form: b, *I. clavata*, *Martyn 379* (M). Verrucate form: c, *I. gardneriana*, *Gardner 3563* (G). Baculate form: d, *I. panamensis*, *Woodson et al. 1685* (MO). e, *I. panamensis*, *Woodson et al. 1685* (US). f, *I. panamensis*, *Gómez 17530* (CR). Bar = 200 μm.

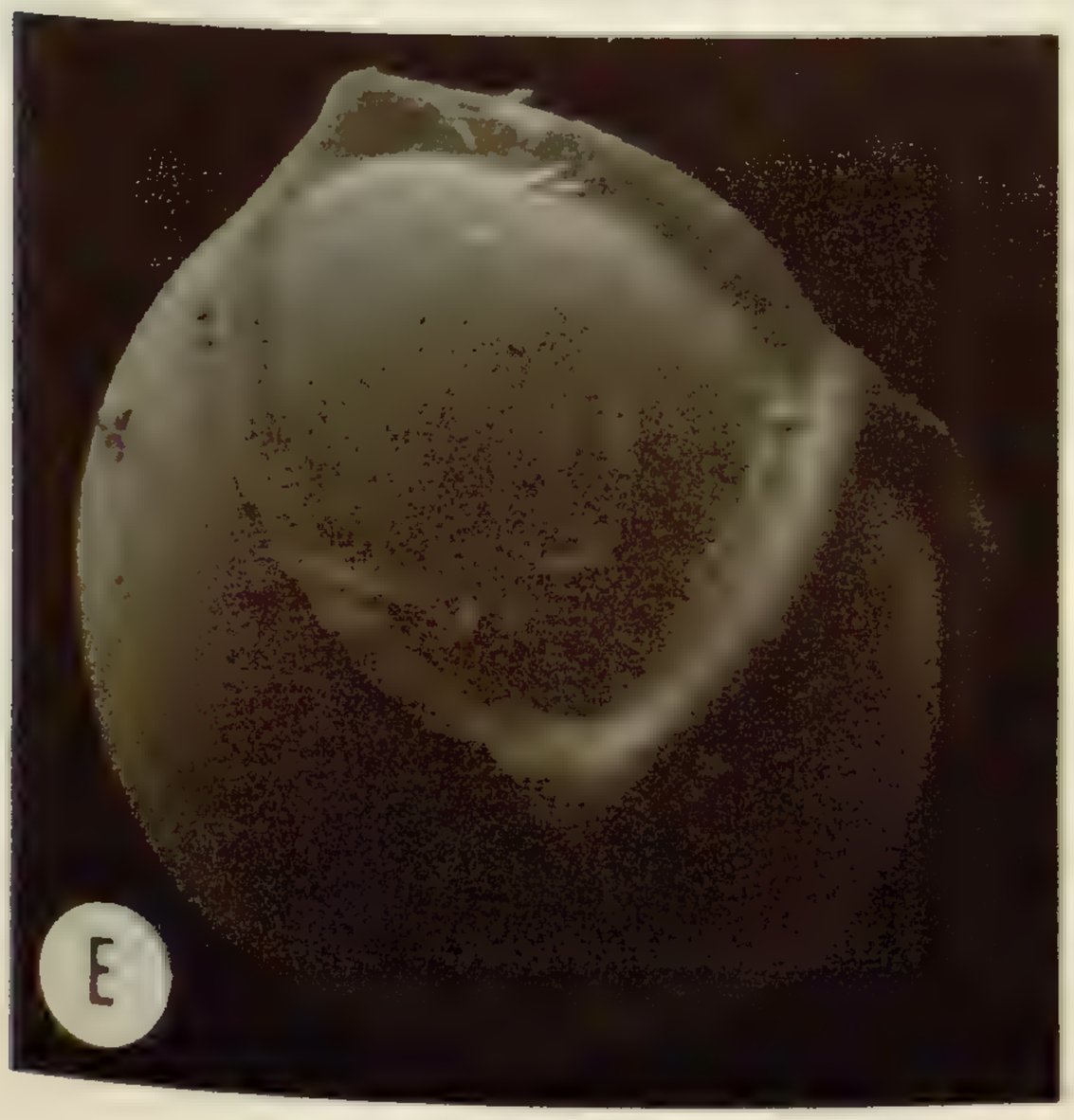
FIG. 3. Megaspore surface morphology in *Isoëtes*. Rugulate form: a, *I. herzogii*, *Hickey 821 & Eshbaugh* (AAU); b, *I. weberi*, *Herter 95840* (NY); c, *I. spec. nov.*, *Chavez 2323* (GH). Cristate form: d, *I. ulei*, *Ule 3533* (CORD). Reticulate form: e, *I. martii*, *Sehnem 14987* (Fuchs' herbarium); f, *I. spec. nov.*, *Spannagel 301* (S). Bar = 200 μm.


A


B

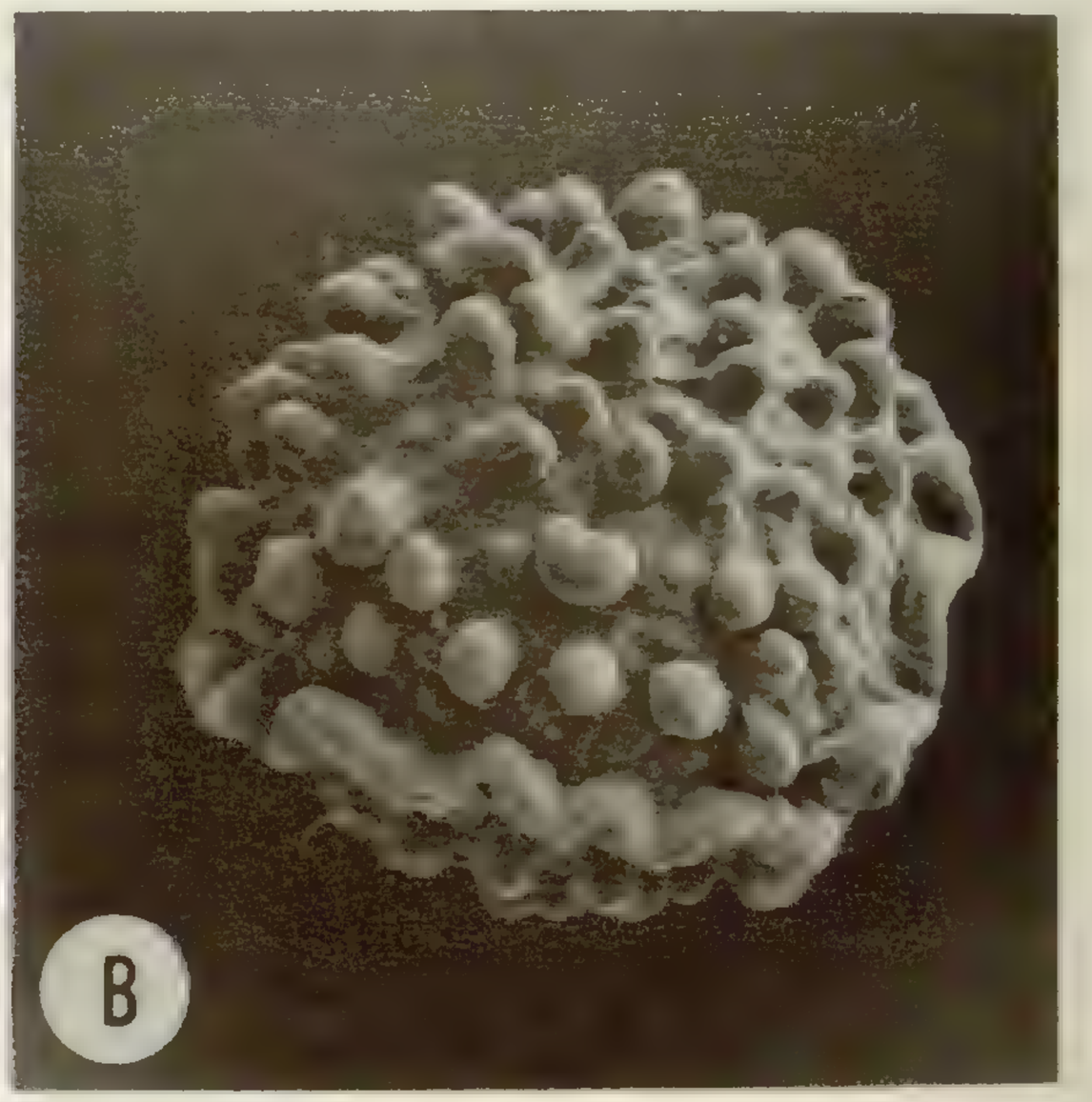

C

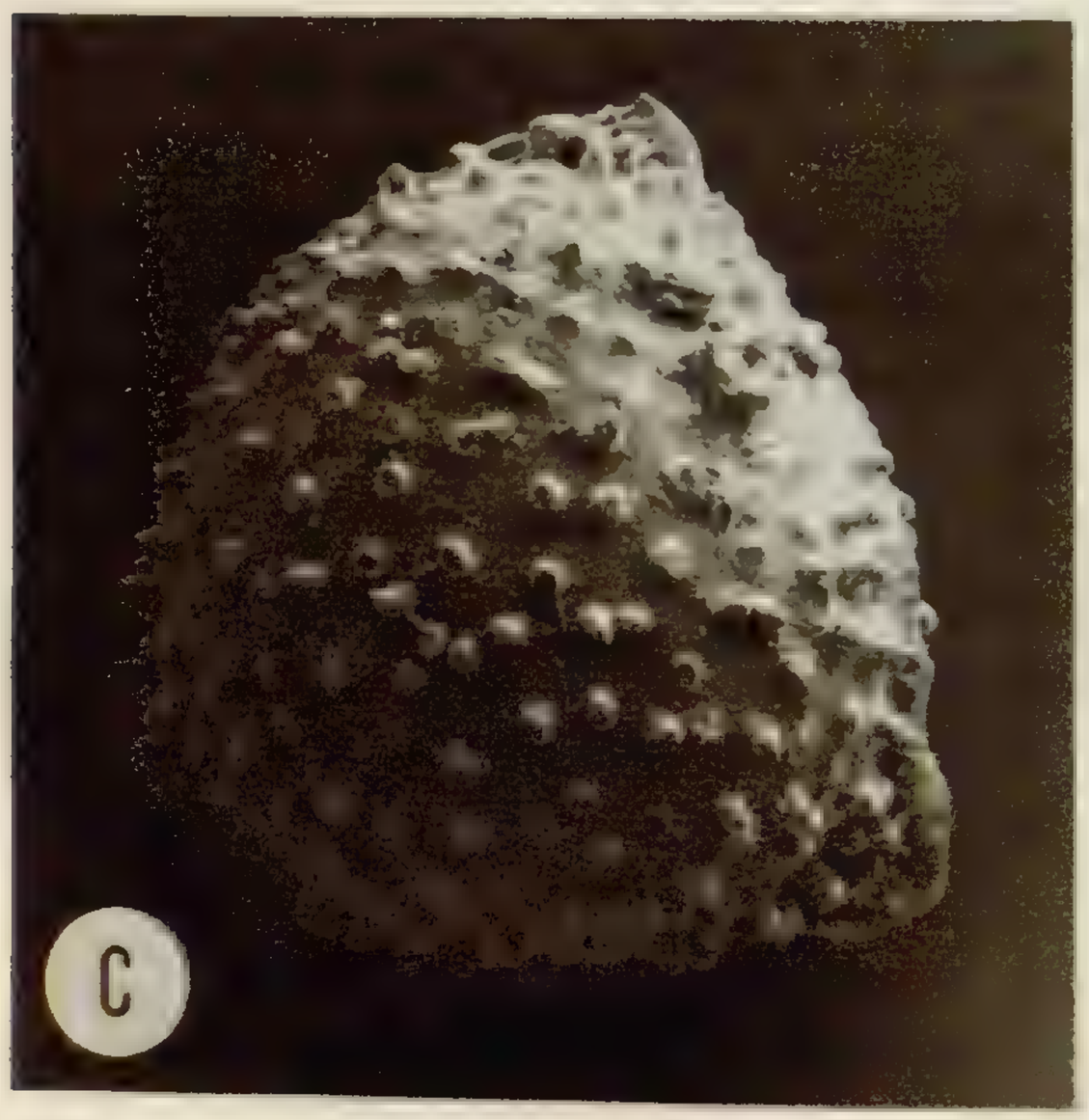

D

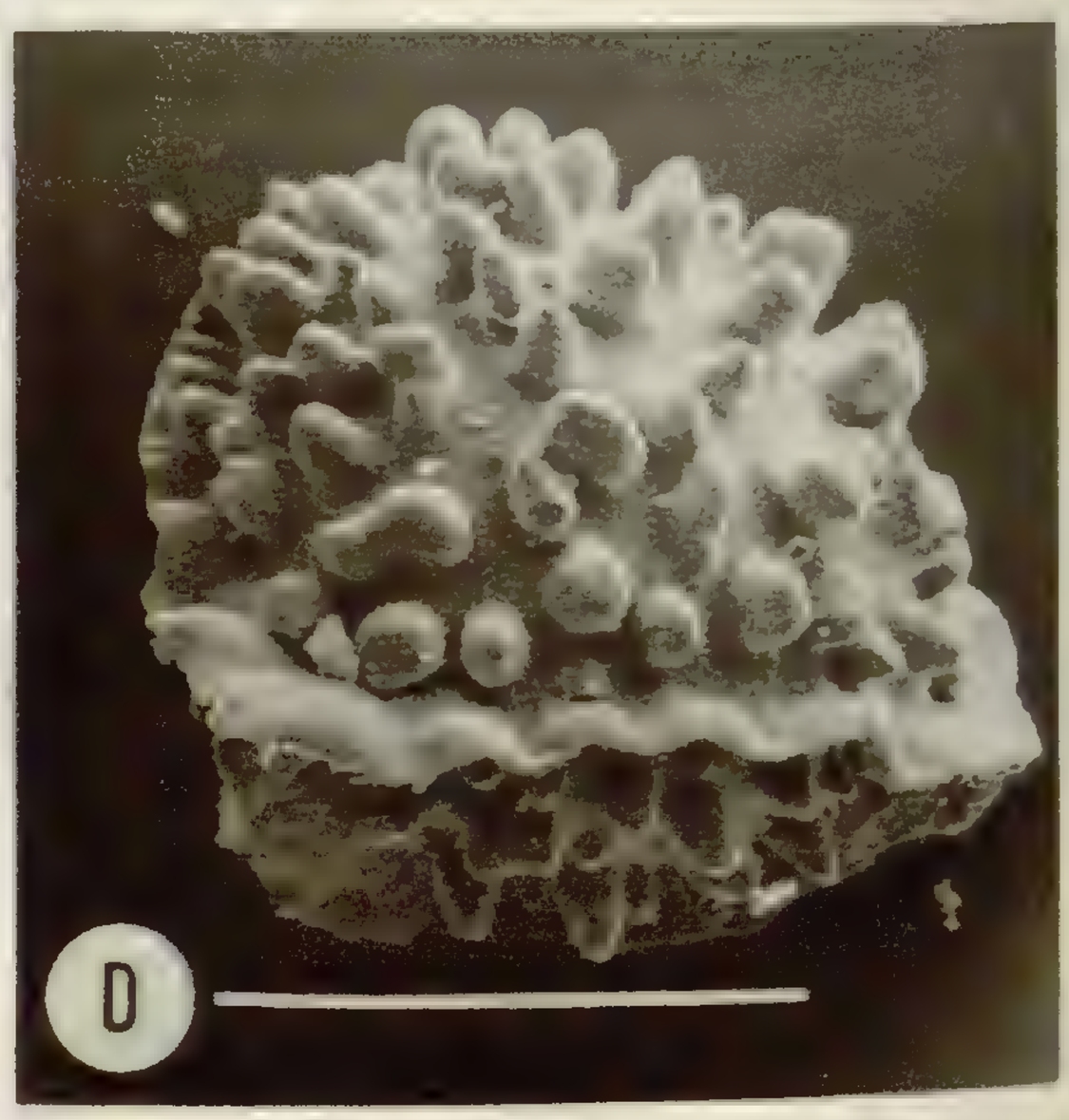

E

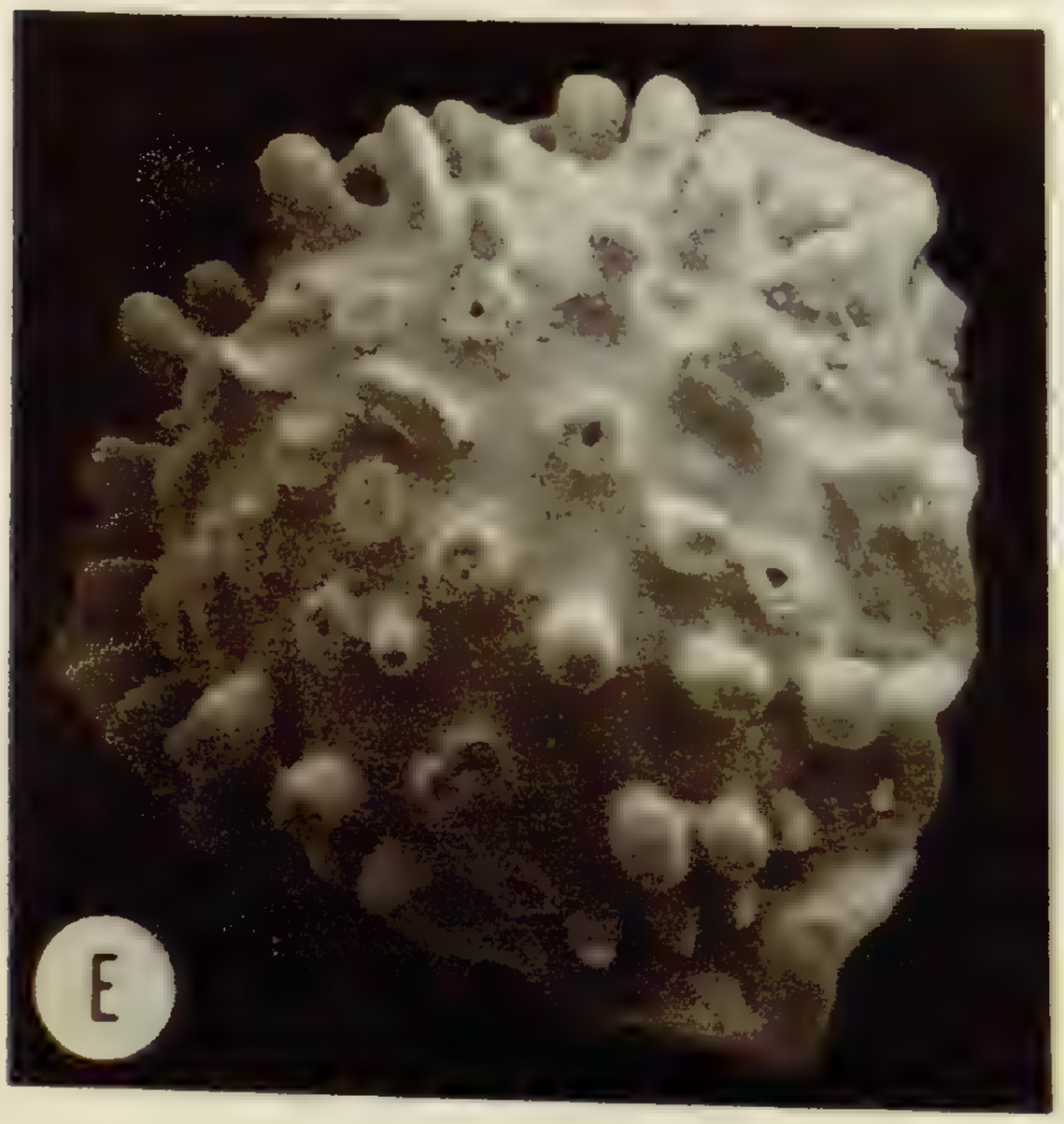
F

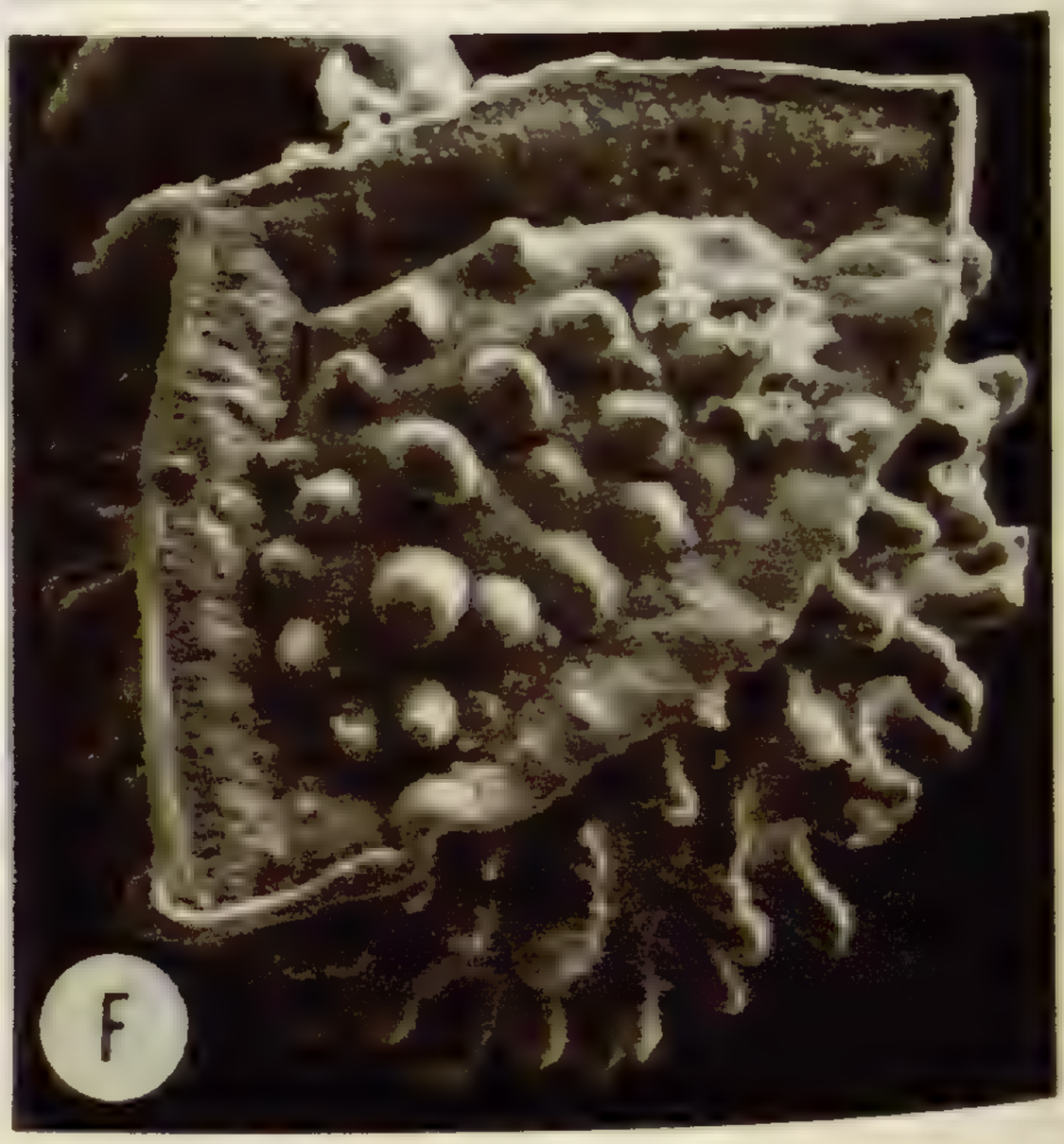
A

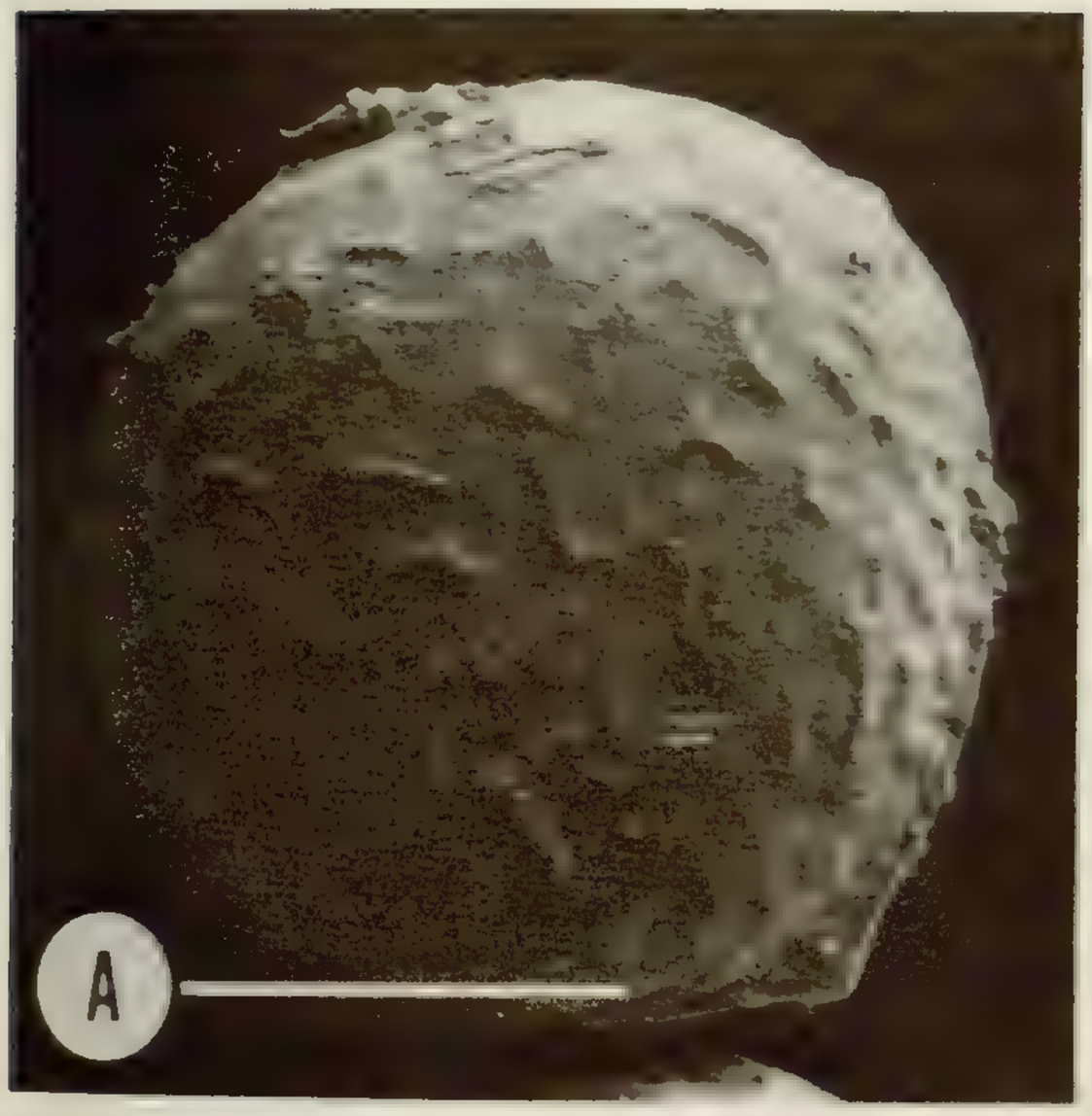

B


C

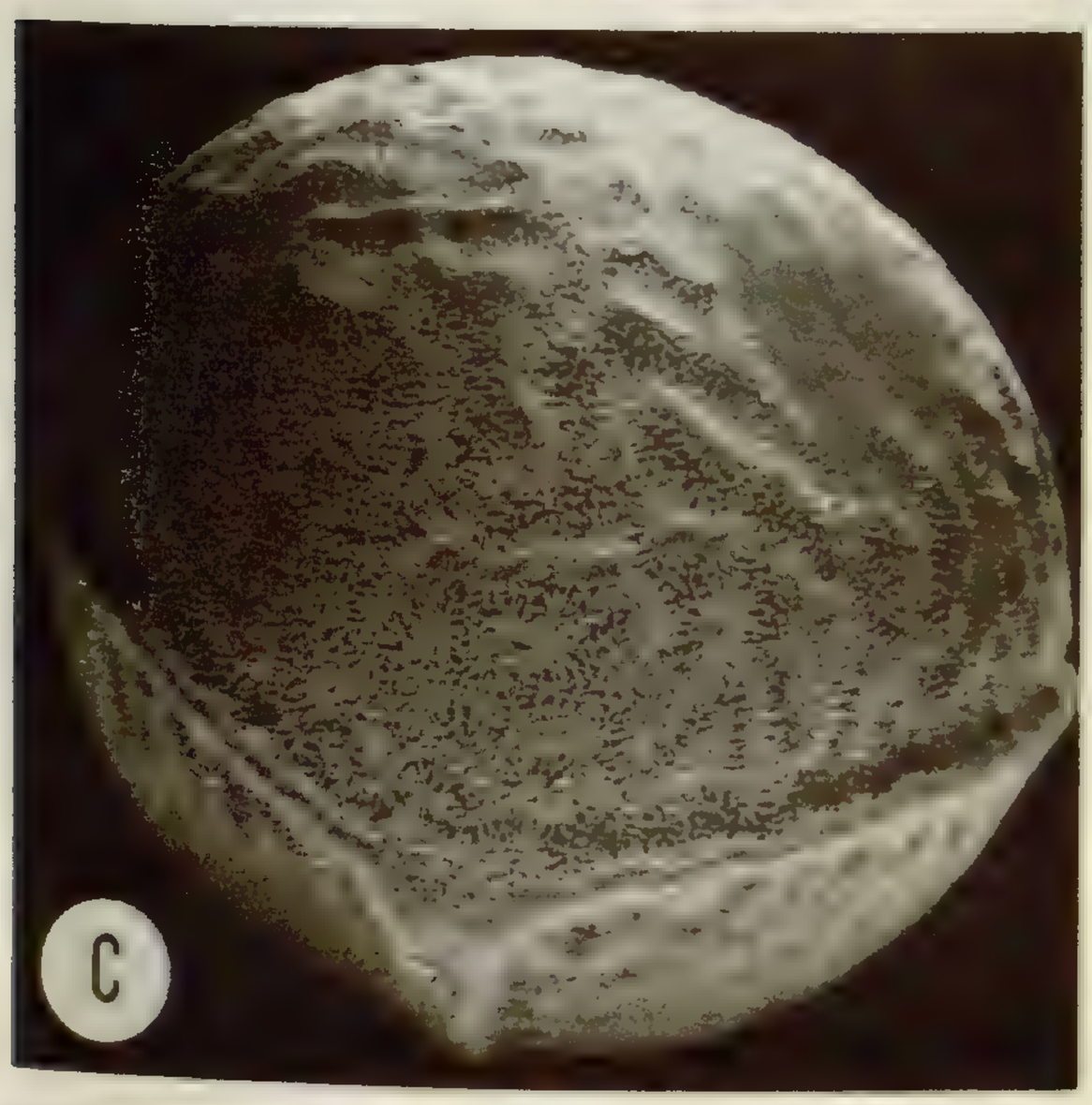

D

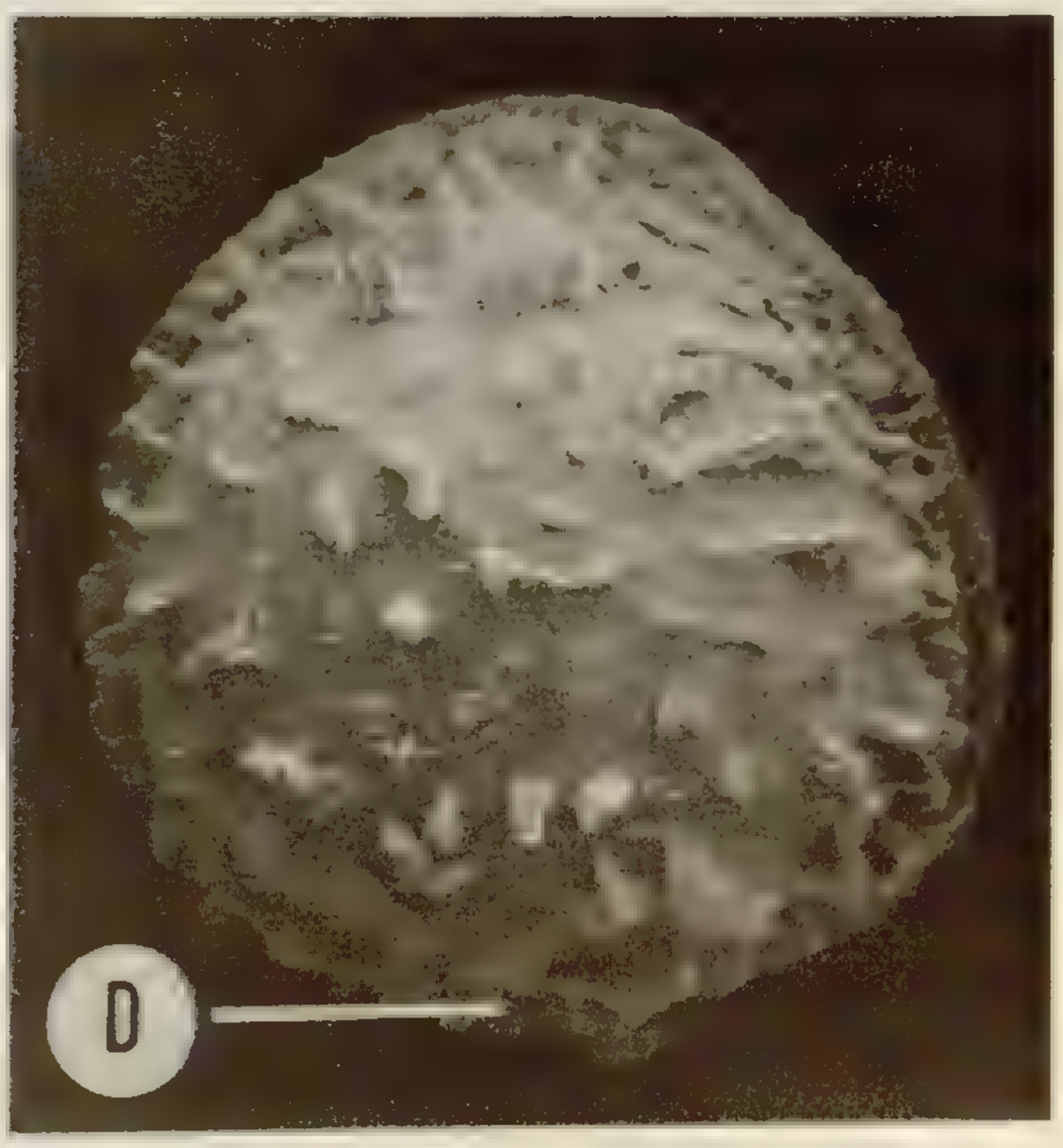

E

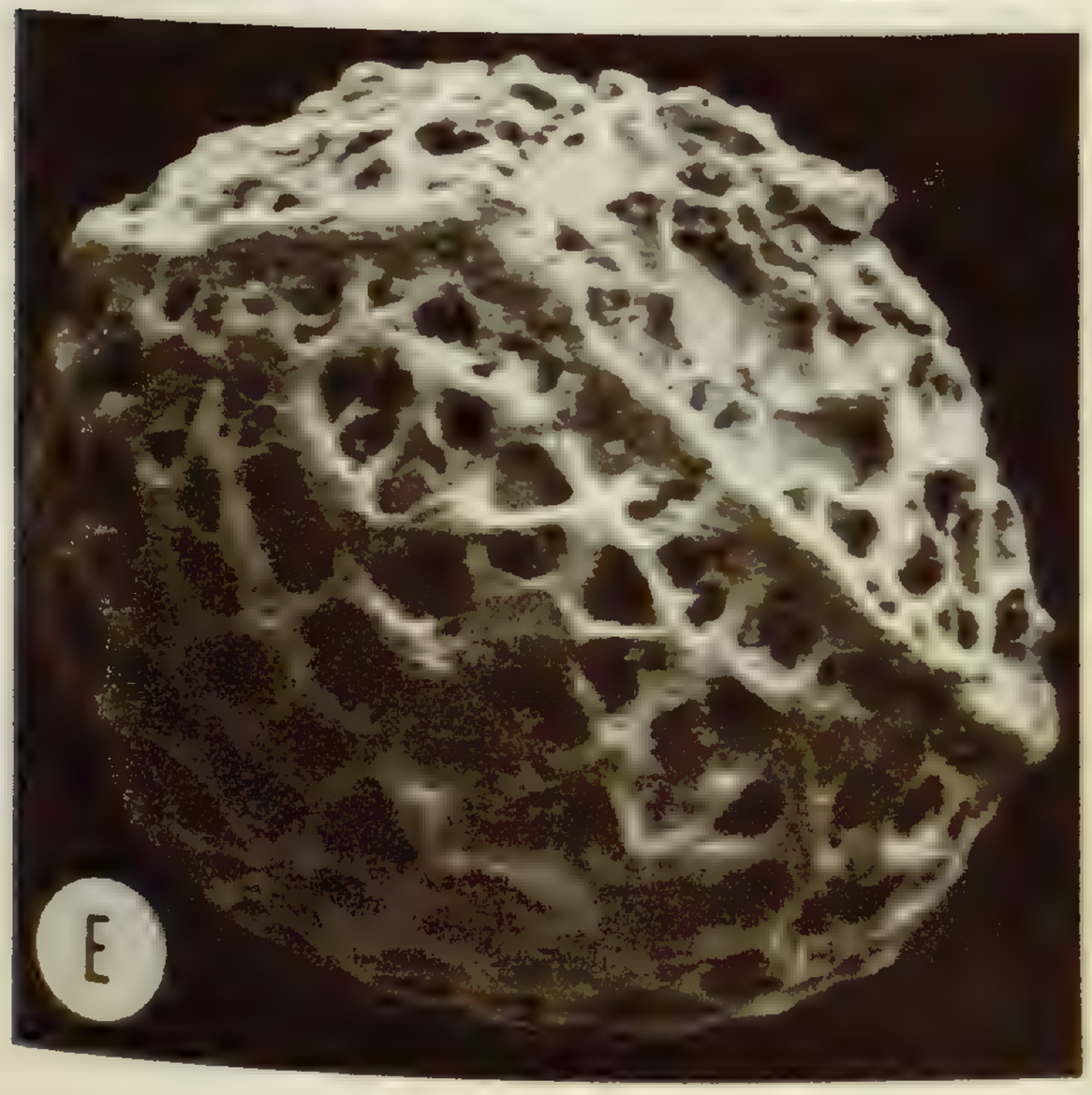

F

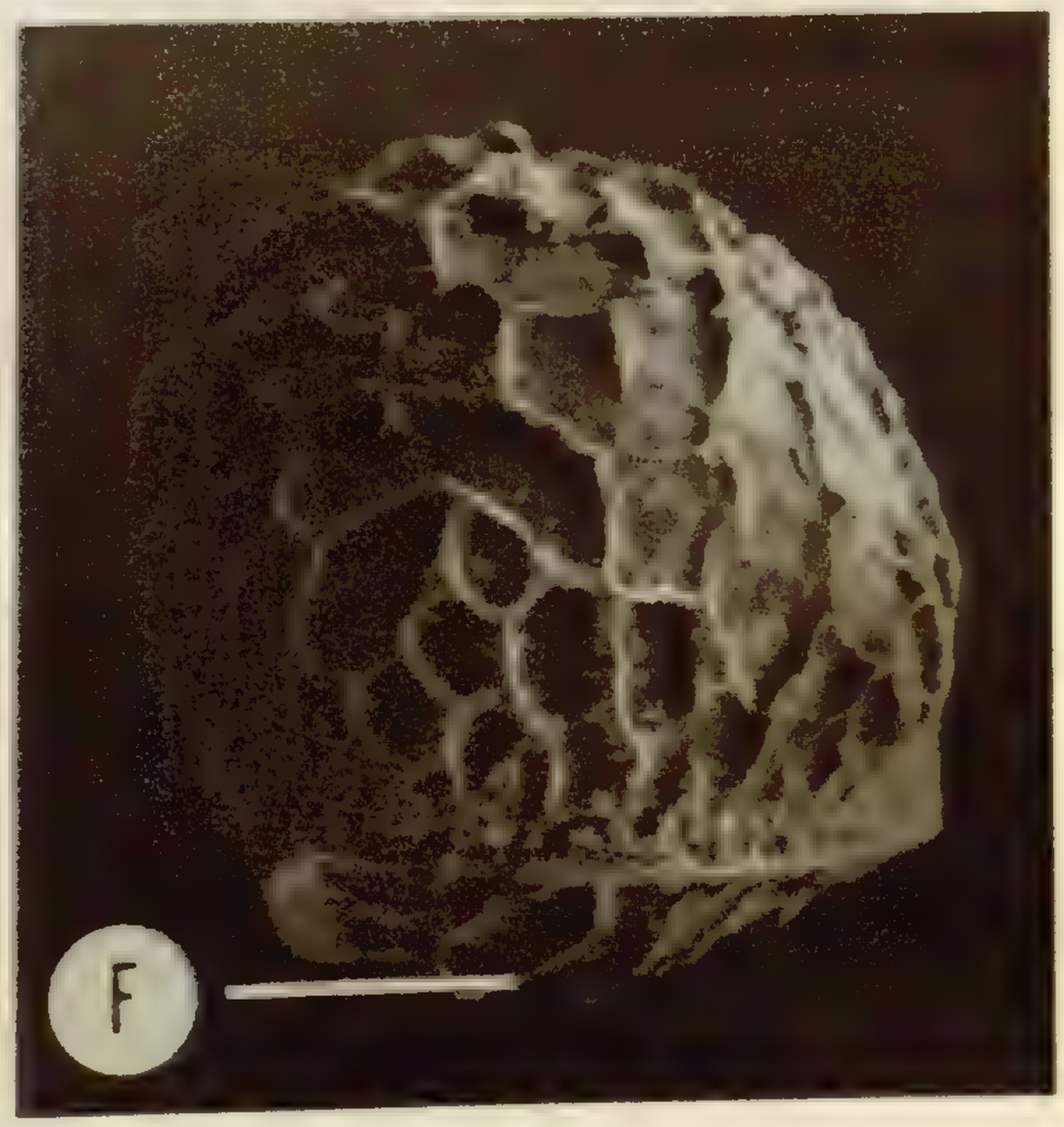

A

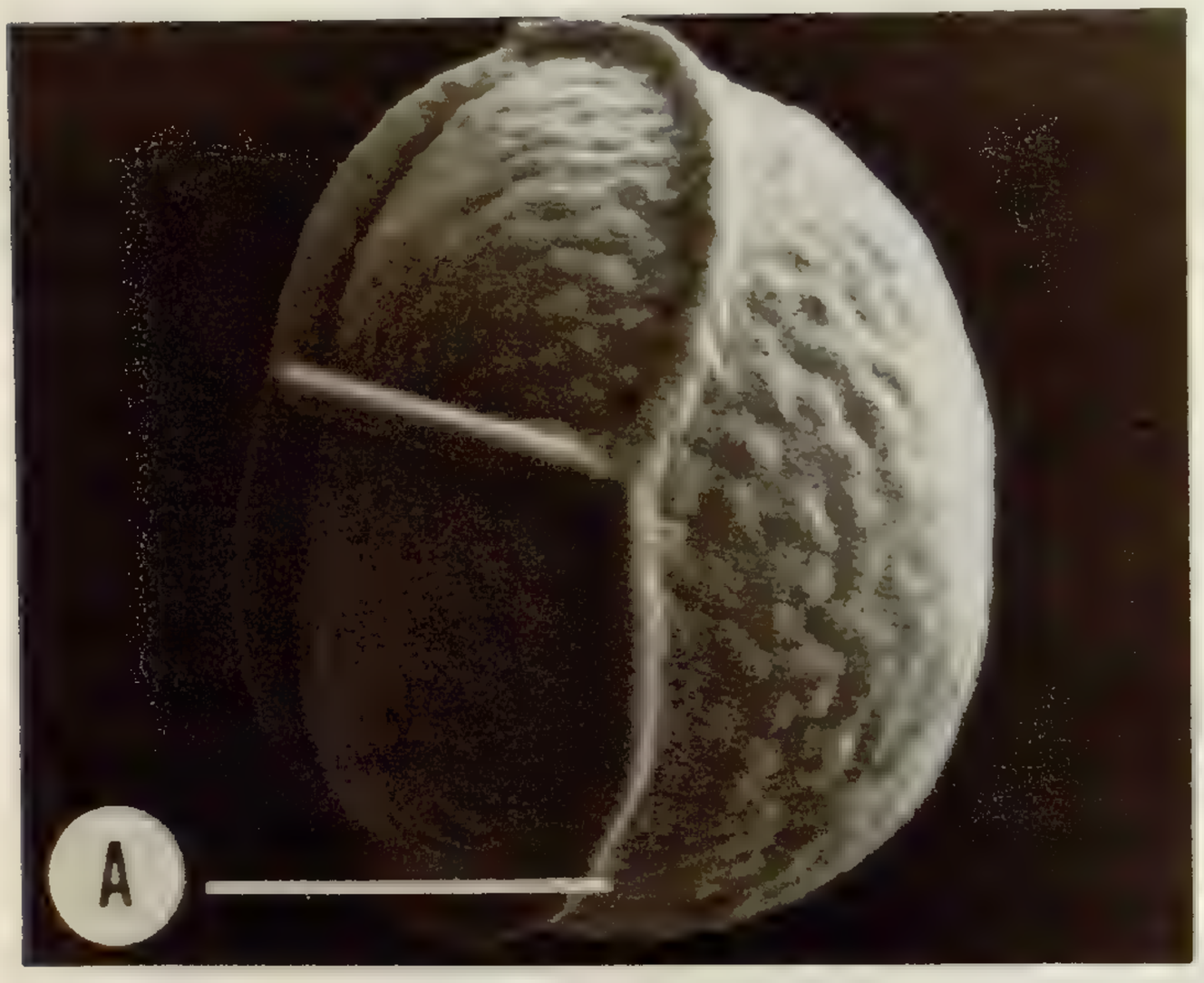
B


C

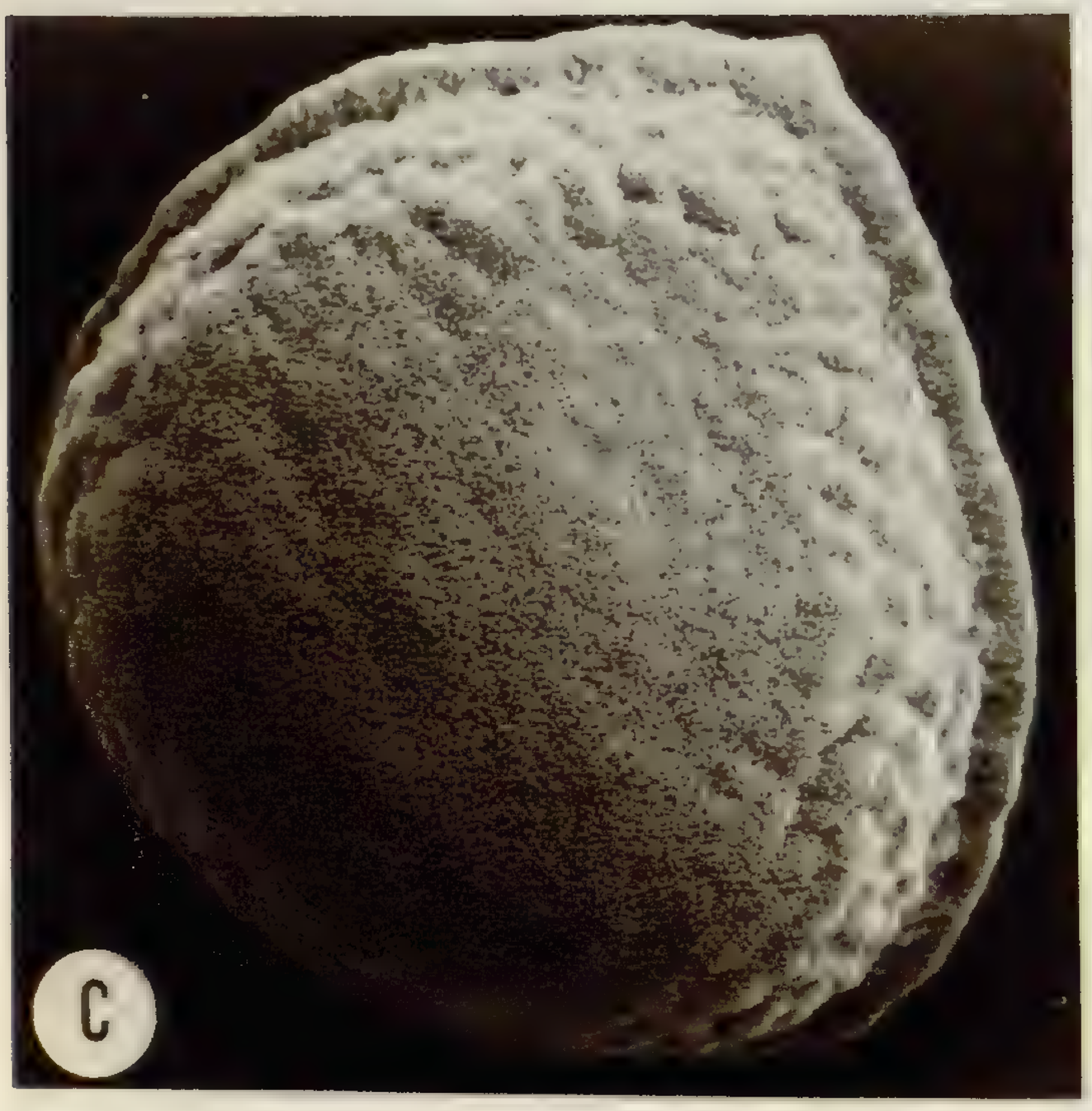

D

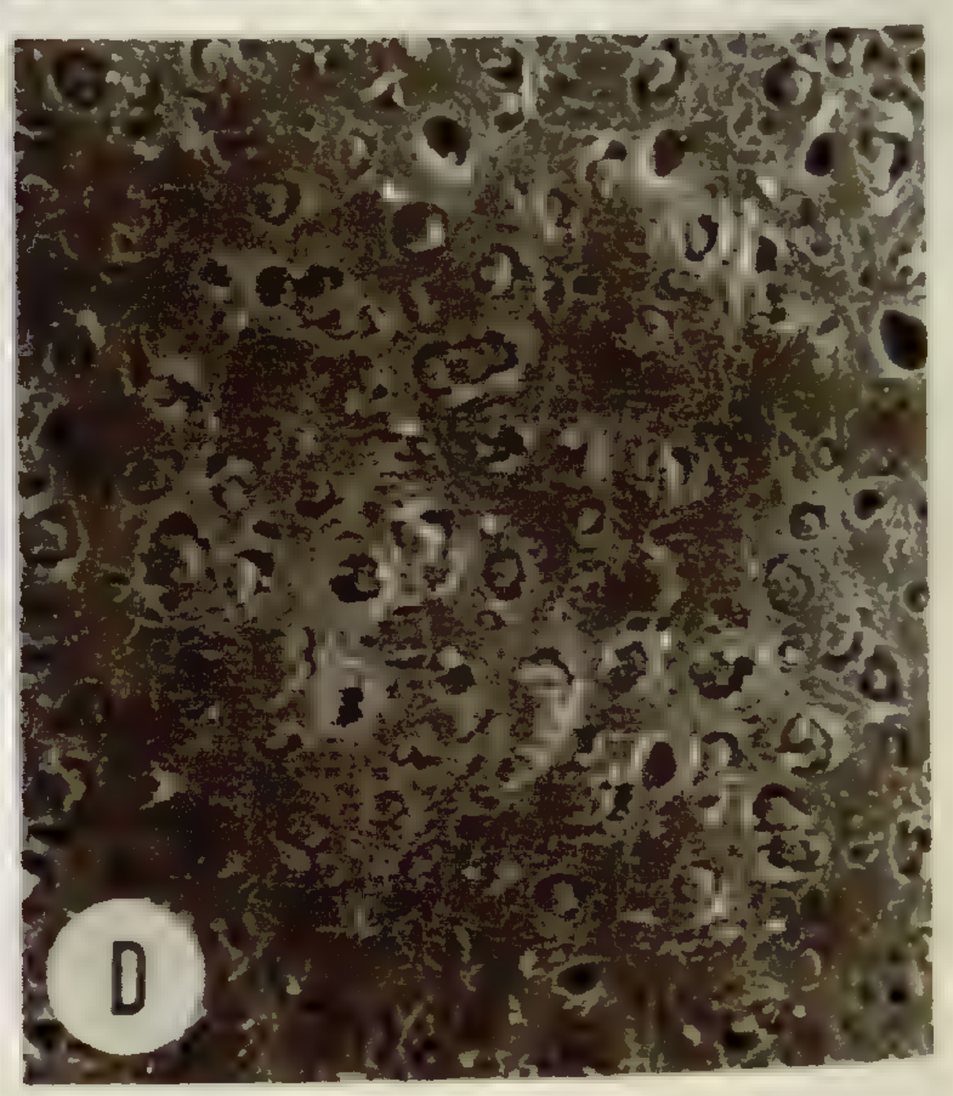

E


F


A

B


C


D

E

F

of Africa. In contrast, the sinuous equatorial ridge (Figs. 2b, d–f) often seen in *I. panamensis*, *I. cubana*, and *I. acadiensis* is variable and unreliable as a species identifying character.

Spore Maturity

While an extensive study of spore development has not been undertaken, a number of developmental changes have been observed which are worthy of discussion. Many of these changes have been previously reported (Boom, 1982; Kott & Britton, 1983) but are important enough to be repeated. As *Isoëtes* megaspores mature, the form and consistency of the perispore (sensu Robert et al., 1973) undergo distinct changes. Initially the perispore is soft and mealy and, when touched, portions of it tend to flake off. With age, the perispore solidifies into a uniform, integral structure. This change in texture has been regarded as the "test" of spore maturity (Boom, 1982; Kott & Britton, 1983). Spore maturity has not been a major problem in *Isoëtes* taxonomy and I know of only one species, *I. saccharata* Engelm., which has been described on the basis of immature spore characters. The real difficulty associated with spore maturity lies in the subsequent identification of specimens. While an ornamentation pattern is apparent fairly early in spore development (Figs. 4a, b), it may be drastically different from that of the mature spore. Developmental changes in ornamentation result from either an initial lack of ornamentation or an obscuring of initial perispore patterns by subsequent wall deposition. This is particularly evident in *I. andicola* (Amstutz) Gómez (Fig. 4) where young spores are weakly pustulate (Figs. 4a, b) and older spores vary from pustulate, to rugulate, to levigate.

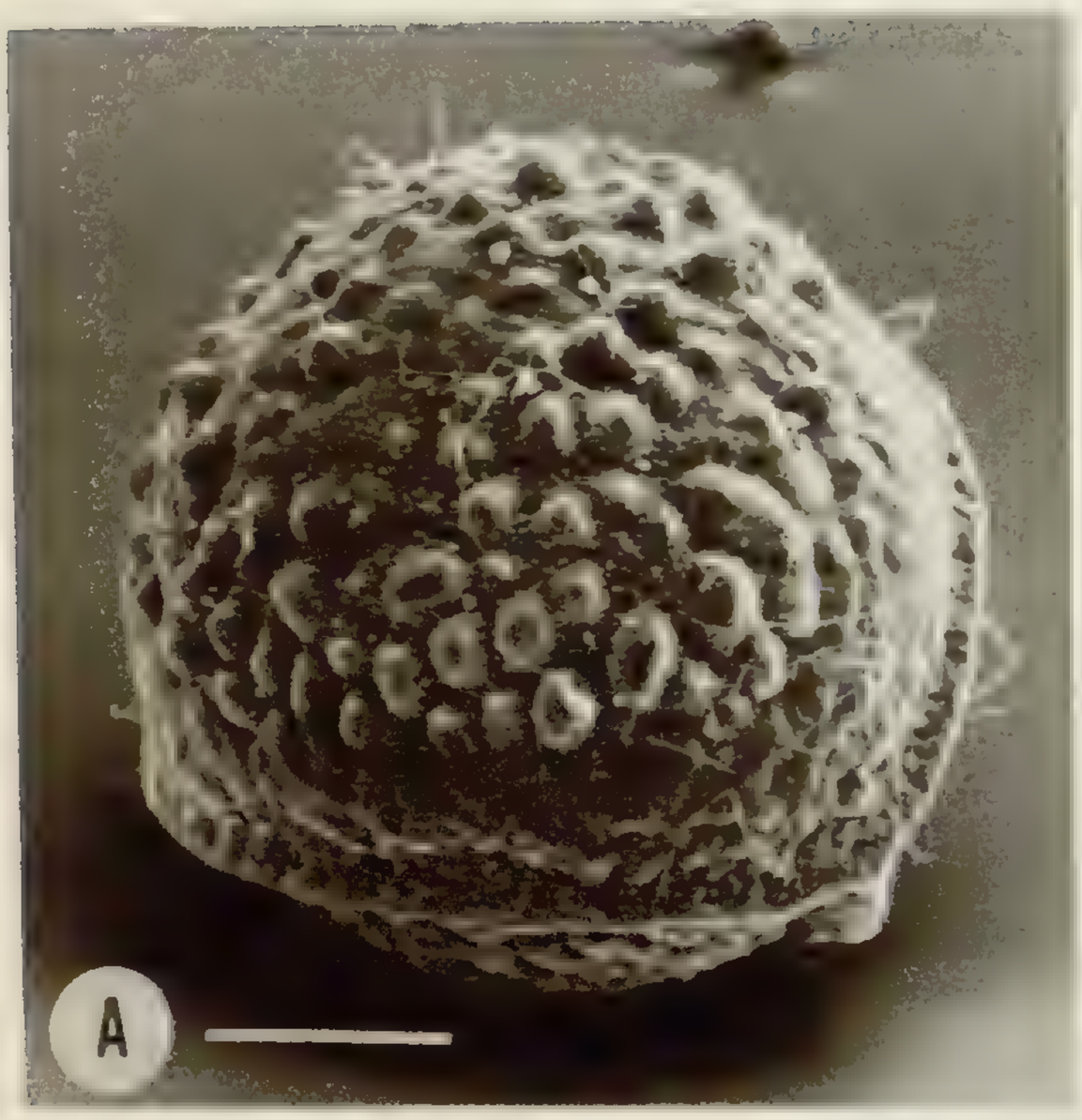
Because the developing perispore is soft, the surface morphology of spores from plants collected early in the growing season is often distorted when pressed for herbarium specimens. Distortion is also possible in specimens where the perispore deposition is complete but where the wall has not completely solidified. In such cases, the ornamentation is evident, but the various protuberances and muri become flattened or bent out of position (Figs. 1f, 2b). Once the perispore hardens, it is brittle and easily damaged (Figs. 2e, 3d). On some spores, such damage can be so extensive as to make it difficult or impossible to interpret clearly the original surface pattern.

Polymorphism

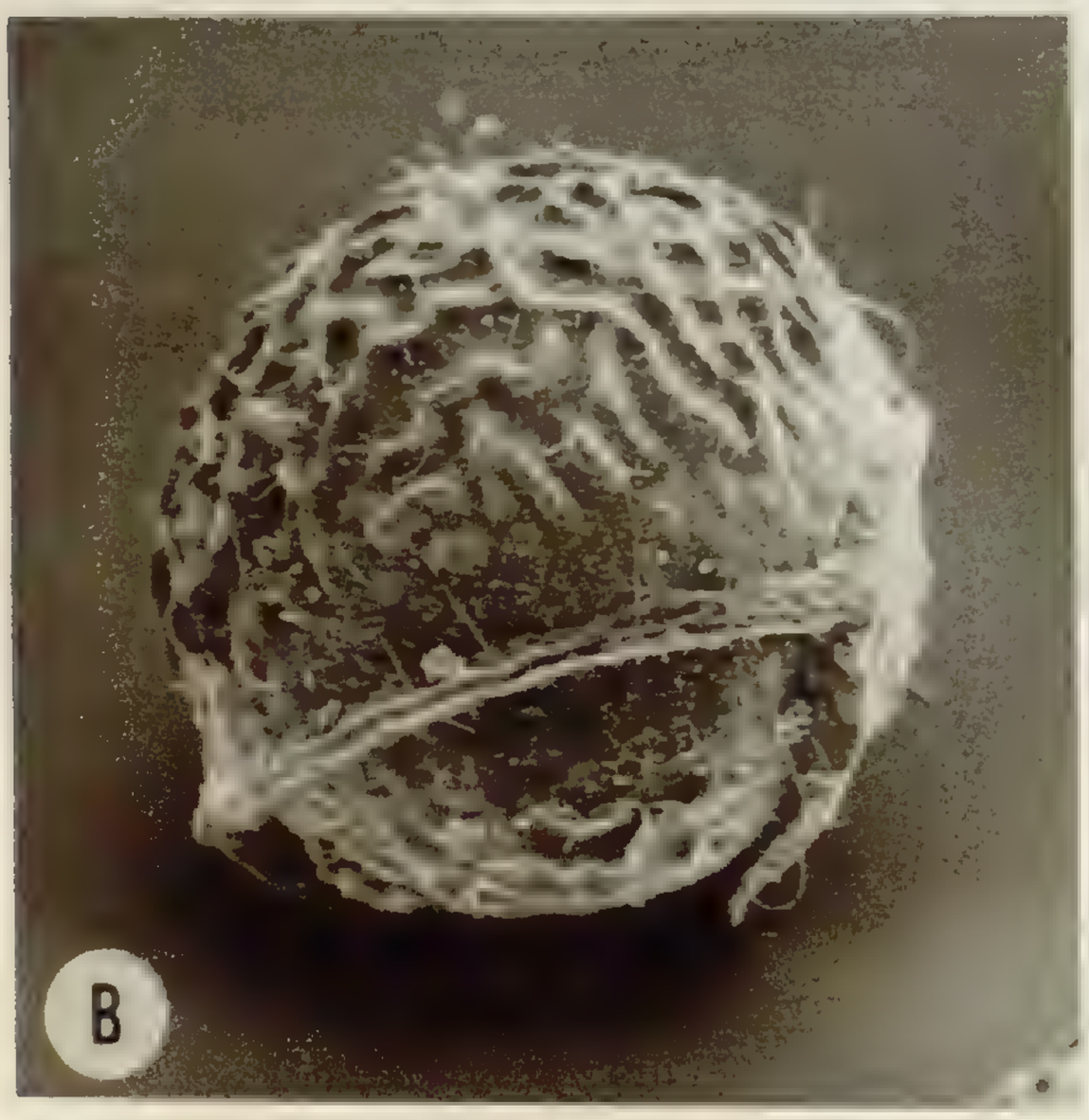
Prior to Pfeiffer's (1922) monograph, megaspore surface morphology was considered so variable as to "furnish no legitimate grounds for classification" (En-

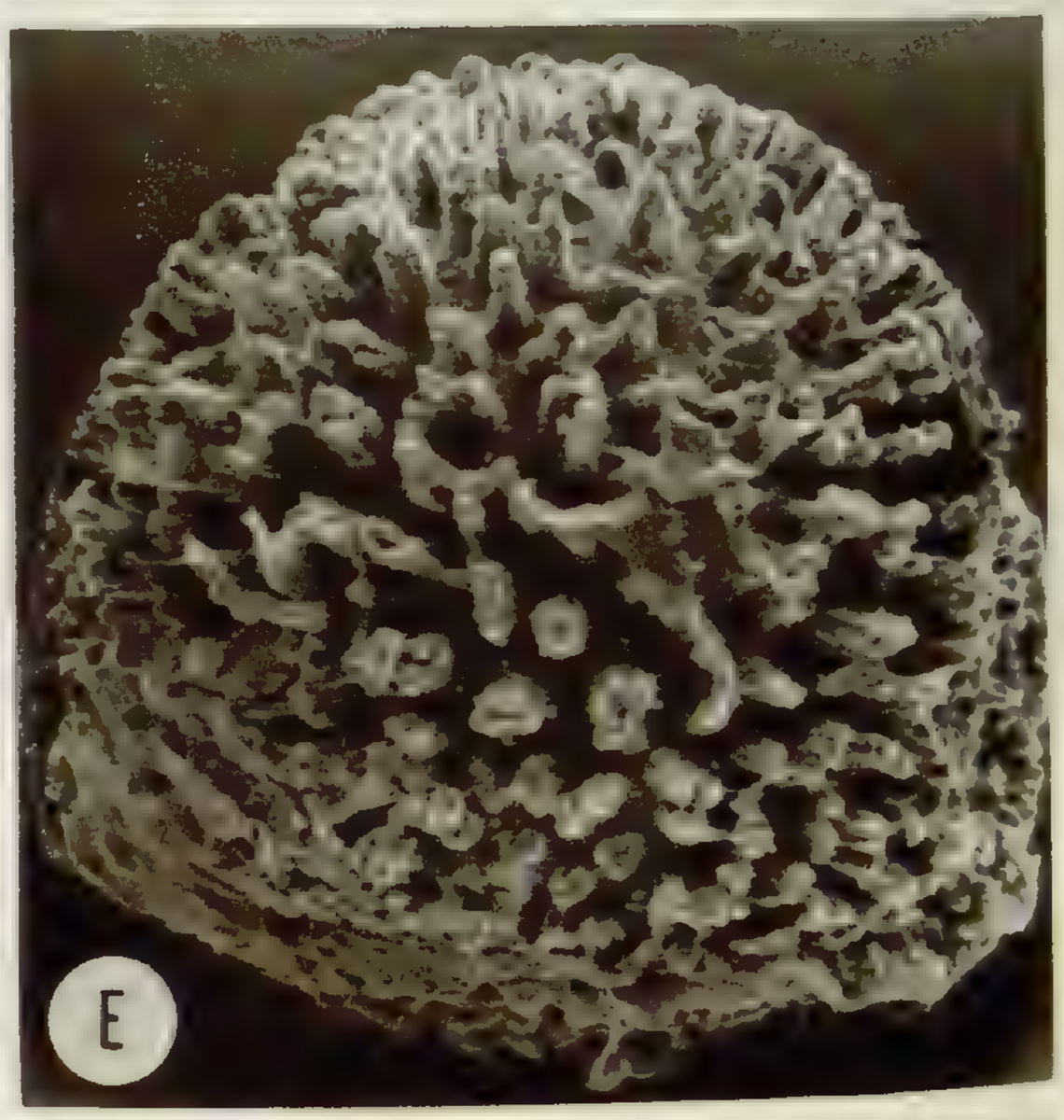
←

FIG. 4. Megaspore surface morphology in *Isoëtes andicola*: a and b, c and d, and e and f are photographs of megaspores taken from successively older sporophylls of *Hutchinson & Tovar* 4244 (GH-spirit material). Photos b, d, and f are high magnification views of the spores shown in a, c, and e respectively. Bars in a, c, and e = 200 μm; bars in b, d, and f = 30 μm.

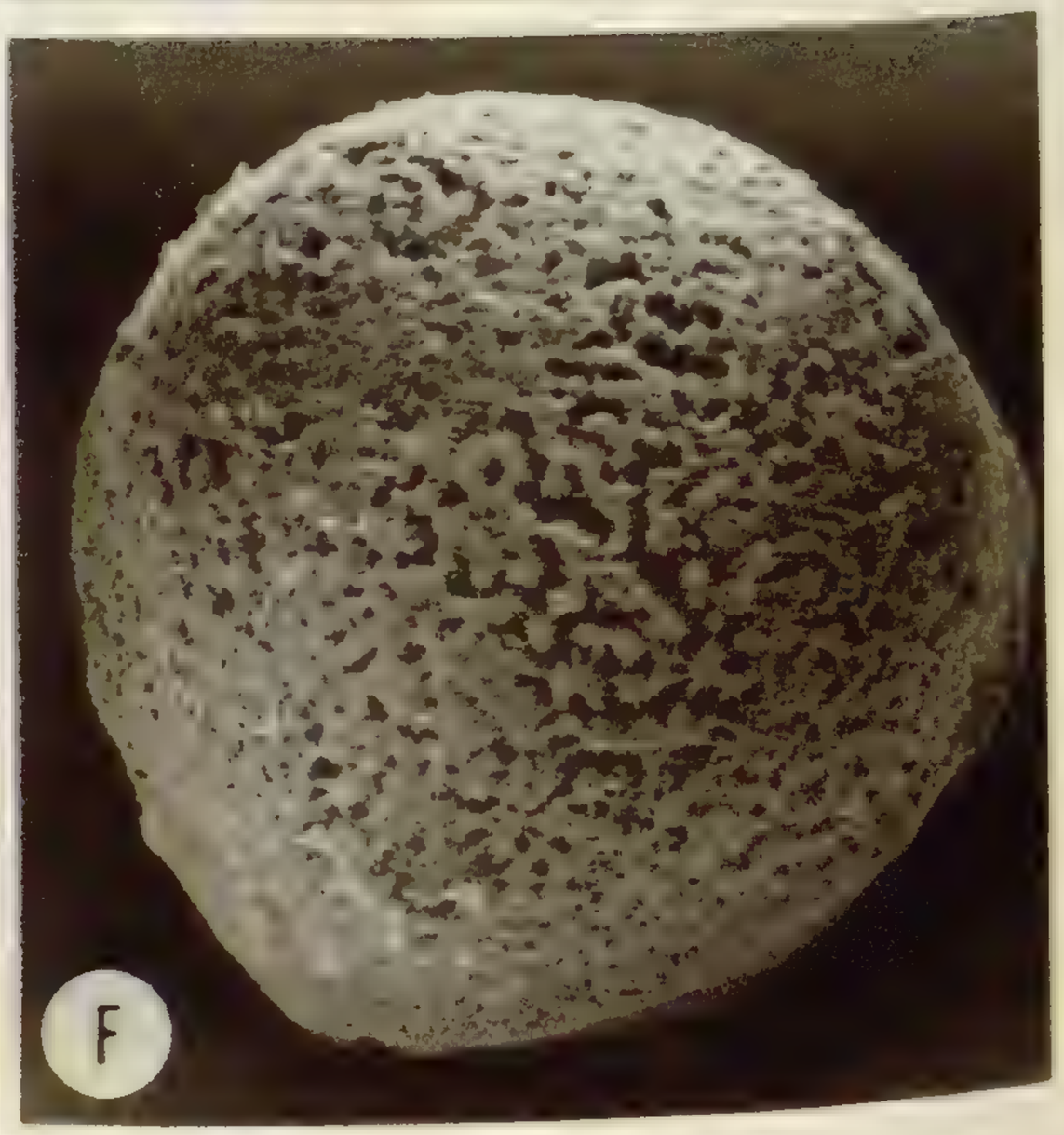

A

D


B


E

C


F

gelmann, 1882). However, since the publication of Pfeiffer's monograph, variability in spore morphology has been largely ignored. In an organism where so many morphological characters have been excluded because of phenotypic plasticity, it is surprising that the variability in the primary character has not been more openly discussed. The following discussions present a brief overview of the more common patterns of infraspecific variation observed in *Isoëtes* megaspore ornamentation. Such variation falls into two major categories: clinal and non-clinal.

Clinal variation.—Perhaps the first recorded example of the recognition of clinal variation is that given by Eaton (1900) in a discussion of changes in megaspore ornamentation in *I. engelmannii* along a north–south transect. Recently, Croft (1980) interpreted the species diversity (and megaspore surface morphology) in Papuasian *Isoëtes* to be a result of speciation along an ancient east–west cline. Extensive geographic variation was noted in *I. riparia* by Proctor (1949), but was not interpreted as clinal. The presence of conflicting taxonomic interpretations (Proctor, 1949; Reed, 1965; Kott & Britton, 1983) of this group suggests that additional study is still needed.

Clinal variation in any character, including megaspore surface ornamentation, is neither surprising nor problematic. Thorough documentation of such variation could help us understand modes of speciation in addition to the origins and relationships of spore ornamentation patterns. The generally restricted ranges and the relatively few collections of most *Isoëtes* species account, in part, for the lack of documentation of otherwise obvious clinal variation. Clines have also been overlooked due to rather narrow species circumscriptions and the lack of accompanying discussions of taxonomic relationships. A careful analysis of the morphology of these narrowly circumscribed species frequently leads to a reinterpretation of their identity as members of subtle continua (Hickey, 1985).

In the Neotropics, geographic and elevational clines are common. *Isoëtes storkii* provides an excellent example of an elevational/ecological cline. This species is endemic to Costa Rica and is known from only three localities, each of which differs in mean temperature, mean daily solar radiation, and elevation (Cox & Hickey, 1984). Individuals from these three populations show intergradation in spore morphology from weakly echinate-cristate, to echinate, to sub-levigate. Megaspore diameter shows a parallel progression from large to small spores.

In the southern Andes, megaspores of *I. savatieri* vary from rugulate, rarely tuberculate, in the south, to rugulate and rugulate-retate in the north (Figs. 5a–c). *Isoëtes andina* and *I. killipii* of the northern Andes (Ecuador to Venezuela) also show clinal variation (Figs. 5d–f). In the former, megaspores of southern plants are typically densely echinate while those from more northerly popula-

←

FIG. 5. Infraspecific variation in megaspore surface morphology in *Isoëtes*. *I. savatieri*: a, Tuberculate form, *Donat 380* (GH); b, Rugulate form, *Borge s.n.* (GH); c, Weakly rugulate form, *Castellanos 114242* (AA). *I. andina*: d, Sparsely echinate form, *Øllgaard & Balslev 8137* (AAU); e, Densely echinate form, *Øllgaard & Balslev 8782* (AAU); f, Densely cristate form, *Øllgaard & Balslev 8943* (AAU). Bar = 200 μm.

tions have a low, crowded, cristate ornamentation. Spores of *I. killipii* from Venezuela are weakly reticulate to reticulate-cristate while those from Colombia and Ecuador are strongly and distinctly reticulate. In the latter two species there is parallel variation in velum development superimposed upon the variation in megaspore morphology.

Non-clinal variation.—Perhaps more striking, and certainly more difficult to deal with, is the degree of non-clinal infraspecific variation within individual populations, plants, and sporangia. While Pfeiffer (1922) recognized the presence of such variation, she considered it to be too minor to negate the use of megaspore surface morphology as the primary systematic character. Duthie (1929), like Engelmann (1882) and Eaton (1900), was not impressed with the importance of spore morphology and commented on the expression and systematic implications of spore morphology in two new African species, *I. capensis* and *I. stephansenii*. The megaspores of these species were described (Duthie, 1929) as ranging from tuberculate, to rugulate, to retate (as reticulate) within a single population; similar variation within sporangia and on the various faces of individual spores was also described. Spores of the Australian *I. muelleri* A. Br. exhibit a range of surface morphology from tuberculate, to rugulate, to retate (Marsden, 1976). In the Neotropics, examples of infraspecific and intrasporangial variation are common (*I. montezumae*, *I. mexicana* Underw., and *I. ulei* U. Weber). Perhaps the most striking example is that seen in populations of *I. andicola* from west-central Bolivia where a single sporangium may contain pustulate, rugulate, and levigate megaspores. This species could be assigned to either sections *Isoëtes*, *Laevis*, or *Palustres*!

Intrasporangial variation may also include differences in spore shape, presence or absence of cellular contents, and spore fusion. These types of variation are often associated with sterile hybrids (Taylor et al., 1985) or plants with unbalanced genomes (Marsden, 1976; Pant and Srivastava, 1962, 1965; Mondal, 1978). While such variation is important, it is beyond the scope of the current paper and the reader is referred to the citations above.

A final pertinent aspect of polymorphism is that dealing with variation in ornamentation on a single spore. It has long been recognized that the distal and proximal faces of a megaspore may exhibit different surface patterns (Boom, 1982; Duthie, 1929; Proctor, 1949; Williams, 1943). For example, megaspores of *I. herzogii* are usually either levigate or tuberculate on the proximal faces but are most commonly rugulate distally. Such variation presents both descriptive and taxonomic problems. In describing the megaspore surface morphology, the patterns observed on both the proximal and distal faces must be compared. A simple description, such as "spores rugulate," is insufficient. Traditionally, when such spores were encountered only the distal pattern was communicated, a situation which often led to errors in identification. Even with spores showing homogeneous ornamentation, the description must be more explicit so as to remove any ambiguity, e.g. "all faces reticulate." The taxonomic consequences of this problem are similar to those encountered with species producing several megaspore types. Despite a lack of biological justification, it has generally been assumed that the distal spore face represents the "official" character state and it is on the

basis of its ornamentation alone that species have been classified. A species like *I. eshbaughii* could be assigned to either sect. *Isoëtes* or *Palustres* on the basis of distal spore morphology, whereas using the proximal ornamentation it could be included only in sect. *Palustres*.

Convergence of Megaspore Surface Features

A high degree of convergence in surface morphology is suggested by a consideration of the form and distribution of megaspore characters as discussed above. Further support for this conclusion, reviewed below, is derived from character compatibility, morphocline analysis, parsimony assessment, infraclade analysis, and hybridization studies (Hickey, 1985).

The presence of morphologically similar megaspore types in many unrelated species is strong evidence that many ornamentation patterns are convergent. For instance, levigate megaspores are found in *I. andicola*, *I. hypsophila* Hand.-Mazz., *I. hopei* Croft, in the *I. montezumae* complex, the *I. herzogii* complex, the *I. ecuadoriensis* complex, and others. Comparative data from geographic distribution, subula shape, velum coverage, labium and ligule morphology, stomate distribution, scale and peripheral fiber development, microspore ornamentation, habitat data, and cuticle development indicate that these species and species groups represent evolutionarily distinct lineages. This conclusion is further supported by an examination of the levigate spore surface. In the *I. montezumae* and *I. herzogii* species complexes, the spore wall is made up of a thick, evenly deposited exospore with little or no perispore externally whereas in the *I. ecuadoriensis* and *I. lechleri* complexes the exospore layer is thin and the perispore is well-developed. Similar examples of homoplasy are found among species with rugulate megaspores (*I. savatieri*, *I. capensis*, *I. andicola*, *I. hieroglyphica*, etc.), reticulate megaspores (*I. martii* A. Br. ex Kuhn, *I. killipii*, *I. engelmanii*, etc.), and echinate megaspores (*I. echinospora* Dur., *I. storkii*, and *I. andina*).

Convergence as an explanation for the presence of similar megaspores among various taxa is also supported by the repeated, parallel occurrences of morphologically distinct megaspore types within species, within individual plants, and within individual sporangia. The tuberculate-rugulate-retate morphocline, for example, is seen in *I. savatieri*, *I. capensis*, *I. stephansenii*, and *I. muelleri*, and a tuberculate-rugulate-levigate morphocline in *I. andicola*, *I. montezumae*, and *I. herzogii*. Additional examples of repeated morphoclines in apparently unrelated taxa are abundant. It is highly improbable that taxa sharing identical or even similar patterns of megaspore polymorphism evolved from the same polymorphic ancestors. Such a scenario would demand an untenable degree of convergence, parallelism and reduction in virtually every other character.

The lack of correlation between megaspore character states and other morphological characters was first demonstrated by Berthet and Lecocq (1977). They showed a lack of significant correlation between megaspore and microspore ornamentation. In my own studies, similar comparisons between megaspore ornamentation and both vegetative and microspore characters failed to show sig-

nificant correlations. The strongest correlations found have been between tuberculate megaspores and either echinate microspores or the absence of a well-developed velum. Not only are these correlations weak, all three of the character states in question are primitive and hence point to a grade level relationship only. There is no evidence to suggest that taxa sharing these primitive states represent an independent evolutionary lineage.

Additional evidence for convergence is found among the diversity of megaspore types produced by species within known monophyletic assemblages (as defined by uniquely derived character conditions). For example, the peculiar phyllopodia of certain African and European species represents a derived character state relative to the sclerified scale (Hickey, 1986a). Species posessing this derived condition show tuberculate, rugulate, and retate patterns of ornamentation. It is postulated (Hickey, 1985, 1986a) that the rugulate and retate spore types evolved subsequent to the divergence of this clade and that similar spore types in other species alliances are not homologous. Even more striking in its megaspore variation is the Neotropical clade (which includes, among others, *I. panamensis*, *I. gardneriana*, and *I. montezumae*) characterized by a unique, trigonal subula (Hickey, 1985). The megaspore variation in this lineage ranges from tuberculate as the primitive state, to the more derived baculate, rugulate, levigate and pustulate conditions. As in the preceding example, the presence of similar spore types in other evolutionary lineages is considered to be the result of convergence.

A final piece of evidence for "convergence" of spore morphology comes from recent hybridization experiments. Boom (1980) and Taylor et al. (1985) have shown that there are no sterility barriers between several species of certain sections of *Isoëtes*. Their data suggest that species from different sections of *Isoëtes* (as defined by spore morphology) are not as isolated as was previously believed. Furthermore, Taylor et al. (1985) suggest that the cristate megaspore pattern seen in *I. eatonii* Dodge, *I. gravesii* A. A. Eaton, and perhaps in *I. riparia*, was derived through hybridization between the reticulate-spored *I. engelmannii* and the echinate-spored *I. muricata*. Their hypothesis is supported by distribution, cytological, enzymatic, and artificial hybridization data. Previously published chromatographic analyses of these taxa (Kott & Britton, 1982) are also compatible with their hypothesis. Considering the large number of polyploid taxa in *Isoëtes* it seems likely that hybridization is a common source of megaspore variation, in particular, as a source of intermediate spore morphologies.

Conclusions

Previous classifications of *Isoëtes* have been based on habitat or inferred habitat preferences or on megaspore surface ornamentation. The former system was found to be unacceptable because of the tremendous plasticity in leaf morphology and the lack of habitat specificity. Acceptance of the megaspore-based classification has been due more to the lack of a viable, alternative classification and to a failure to evaluate critically infraspecific variability and character correlation than to any inherent strengths of that system. The variation described in

this paper indicates that the *Isoëtes* megaspore is not as conservative as some have suggested (Kott & Britton, 1983). To the contrary, evidence is presented which suggests that megaspore variation is at least as plastic as vegetative characters.

The reliability of any character is supported by corroboration with other characters that circumscribe the same taxa (that is, character correlation and its corollary, the lack of incompatibility with other characters). Little consideration has been given to character compatability studies in *Isoëtes* as a means of supporting or refuting the evolutionary relationships among species as indicated by megaspore morphology. The isolated attempts to examine a wider assemblage of characters are especially noteworthy because they have shed light on the role of megaspore morphology in classification. The works of Wagner (1964), Evans (1968), and Boom (1982) indicate that megaspore characters, *when used in concert* with phenology and habitat preference, are useful for delimiting natural species alliances. Likewise, Hickey has shown that multiple character analyses using vegetative features alone or in concert with either habitat data or megaspore morphology provide a basis for lower (1986b) and higher (1986a) taxonomic delimitations.

Patterns of variation in megaspore ornamentation indicate that surface morphology is extremely liable to convergence. The evolutionary causes for such convergence are unknown but may be the result of: a) parallel responses to particular habitat requirements or environmental stimuli (such as shortened growing seasons), b) inherently limited genetic potential (which canalizes morphological potential), c) selection for synaptosporic dispersal mechanisms (see Kramer, 1977), d) stochastic events, or e) hybridization.

At present, there are insufficient data to advance an infrageneric classification alternative to that based on megaspore morphology. It is first necessary to continue work on a regional level by circumscribing natural species groups and species alliances. Such analyses should examine the variation, polarity, and evolution of the definitive characters. Individual character states must be strictly defined and, whenever possible, convergences identified. The reclassification of megaspores presented in this paper represents a first step in this process by clearly defining the primary states of this character and describing the major sources and patterns of variation associated with those states.

Acknowledgments

I thank Gregory J. Anderson, Anne Bruneau, Jerry Gastony, Carl Taylor, and Rolla and Alice Tryon for their suggestions and criticisms during the preparation of this manuscript. This study was supported in part by two Sigma Xi grants (Hickey), a University of Connecticut Research Grant (Anderson), and NSF Grant BSR 82-07125 (Anderson).

Literature Cited

Baker, J. G. 1880. A synopsis of the species of *Isoetes*. J. Bot. 18:65–70, 105–110.

Berthet, P. and M. Lecocq. 1977. Morphologie sporale des espèces françaises du genre *Isoetes* L. Pollen & Spores 19:329–349.

BOOM, B. M. 1980. Intersectional hybrids in *Isoëtes*. Amer. Fern J. 70:1–4.
———. 1982. Synopsis of *Isoëtes* in the southeastern United States. Castanea 47:38–59.
BRAUN, A. 1864. Les espèces d'*Isoëtes* de l'Ile Sardaigne. Ann. Sci. Nat. Bot. V, 2:306–377.
COX, P. A. and R. J. HICKEY. 1984. Convergent megaspore evolution and *Isoetes*. Amer. Naturalist 124:437–441.
CROFT, J. R. 1980. A taxonomic revision of *Isoetes* L. (Isoetaceae) in Papuasia. Blumea 26:177–190.
DUTHIE, A. V. 1929. The species of *Isoetes* found in the Union of South Africa. Trans. Roy. Soc. South Africa 17:321–334.
EATON, A. A. 1900. The genus *Isoetes* in New England. Fernwort Papers 2:1–16.
ENGELMANN, G. 1882. The genus *Isoëtes* in North America. Trans. St. Louis Acad. Sci. 4:242–280.
EVANS, A. M. 1968. Isoëtaceae. Pp. 8–10 in *Manual of the vascular flora of the Carolinas*, ed. A. Radford et al. Chapel Hill: University of North Carolina.
FUCHS, H. P. 1962. Nomenklatur, Taxonomie und Systematik der Gattung *Isoëtes* Linneaus in geschichtlicher Betrachtung. Beih. Nova Hedwigia 3:1–117.
FUCHS-ECKERT, H. P. 1981. *Isoëtes Palmeri* H. P. Fuchs, eine neue *Isoëtes*-Art des Páramo. Proc. Kon. Ned. Akad. Wetensch. C84:165–174.
HICKEY, R. J. 1985. Revisionary studies of Neotropical *Isoëtes*. Ph.D. dissertation, The University of Connecticut, Storrs.
———. 1986a. The early evolutionary and morphological diversity of *Isoëtes*, with descriptions of two new Neotropical species. Syst. Bot. 11:309–321.
———. 1986b. On the identity of *Isoëtes triquetra*. Taxon (in press).
KOTT, L. S. 1980. The taxonomy and biology of the genus *Isoetes* in northeastern North America. Ph.D. thesis, University of Guelph, Guelph.
KOTT, L. S. and D. M. BRITTON. 1982. Comparison of chromatographic spot patterns of some North American *Isoëtes* species. Amer. Fern J. 72:15–18.
——— and ———. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. Canad. J. Bot. 61:3140–3163.
KRAMER, K. U. 1977. Synaptospory: a hypothesis. A possible function of spore sculpture in pteridophytes. Gard. Bull. Straits Settlem. 30:79–83.
MARSDEN, C. R. 1976. Morphological variation and taxonomy of *Isoetes Muelleri* A. Br. J. Adelaide Bot. Gard. 1:37–54.
MONDAL, M. S. 1978. Spore morphology of *Isoetes coromandelina* L. from Kalyani. Bull. Bot. Soc. Bengal 32:8–12.
MOTELAY, L. and A. VENDRYES. 1882. Monographie des Isoëteae. Actes Soc. Linn. Bordeaux 36:309–406, t. 8–17.
PANT, D. D. and G. K. SRIVASTAVA. 1962. The genus *Isoetes* in India. Proc. Natl. Inst. Sci. India, Pt. B, Biol. Sci. 28:242–280.
——— and ———. 1965. Cytology and reproduction of some Indian species of *Isoetes*. Cytologia 30:239–251.
PFEIFFER, N. E. 1922. Monograph of the Isoetaceae. Ann. Missouri Bot. Gard. 9:79–232.
PROCTOR, G. R. 1949. *Isoëtes riparia* and its variants. Amer. Fern J. 39:110–121.
REED, C. F. 1965. *Isoetes* in southeastern United States. Phytologia 12:369–400.
ROBERT, D., F. FOLAND-HEYDACKER, J. LAROCHE, P. FOUGEROUX, and L. DAVIGNON. 1973. La paroi mégasporale de l'*Isoetes setacea* Bosc ex Delile. Étude en microscopies photonique et électroniques localisation et nature de la silice entrant dans sa constitution. Adansonia, sér. 2, 13:313–332.
TAYLOR, W. C., N. T. LUEBKE, and M. B. SMITH. 1985. Speciation and hybridisation in North American quillworts. Proc. Roy. Soc. Edinburgh 86B:259–263.
TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.
WAGNER, W. H., JR. 1964. Isoëtaceae. Pp. 40–41 in *Guide to the vascular flora of the Carolinas*, ed. A. Radford et al. Chapel Hill: University of North Carolina.
WEST, C. and H. TAKEDA. 1915. On *Isoëtes japonica* A. Br. Trans. Linn. Soc. London, Bot., Ser. 2, 8:333–376.
WILLIAMS, S. 1943. On *Isoetes australis* S. Williams, a new species from western Australia. Part I. General morphology. Proc. Roy. Soc. Edinburgh 62B:1–8.

American Fern Journal 76(1):17–20 (1986)

Active Enzymes from Megaspores of *Marsilea* and *Regnellidium*

DOUGLAS E. SOLTIS and PAMELA S. SOLTIS
Department of Botany, Washington State University, Pullman, WA 99164-4230

Herbarium specimens have, of course, long been invaluable sources of morphological data. More recently, they have also been utilized in chemosystematic studies (e.g. Anderson & Øvstedal, 1983; Giannasi & Niklas, 1981). Secondary compounds, such as flavonoids, are stable and are not degraded following desiccation. Hence, herbarium specimens provide a wealth of potential data in such chemical analyses. However, herbarium specimens apparently have not been utilized in applications of the electrophoretic technique because living material is a prerequisite. The Marsileaceae offers an unusual opportunity to utilize herbarium specimens in electrophoretic analyses. For example, Johnson (1985) demonstrated that 99- to 100-year-old sporocarps of *Marsilea oligospora* still produced functional megaspores and microspores. To determine whether sporocarps also provide a potential source of isozyme data, we initiated an electrophoretic investigation.

MATERIALS AND METHODS

Sporocarps were obtained from specimens (WS) of *Marsilea vestita* and *Regnellidium diphyllum*, scarified with a razor blade, and soaked in water to effect germination. Sporocarps were obtained from the following specimens: *Ownbey* s.n., greenhouse material (*Regnellidium diphyllum*); *Old* s.n., Harney Co., Oregon (*Marsilea vestita*); *Davis 212*, Grant Co., Washington (*Marsilea vestita*). After 24 hours the sorophore (in the case of *Marsilea*) and sporocarp wall were removed and the spore suspension filtered through a Buchner funnel under suction. Megaspores (25–50) were then scraped into a small porcelain mortar. In the case of *Regnellidium*, care was taken to ensure removal of all gelatinous material from the megaspores. Several sporophytes of *Marsilea vestita* were also examined electrophoretically and the banding patterns obtained compared to those observed for megaspores.

The megaspores from a single sporocarp were ground with a glass rod in a small drop of the tris-HCl grinding buffer-PVP solution of Soltis et al. (1983). Sporophytes of *Marsilea vestita* were prepared as previously outlined (Soltis et al., 1983). For both sporophyte tissue and megaspores, the ground plant material was absorbed onto filter paper wicks and starch gel electrophoresis conducted following the general methods of Soltis et al. (1983). The following enzymes were examined: alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), colorimetric esterase (EST), fluorescent esterase (FE), glyceraldehyde-3-phosphate dehydrogenase ([NAD]G3PDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), phos-

phoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI).

For all enzymes examined, a 12.5% starch gel was employed. ADH, AAT, EST, FE, LAP, ME, PGI, and TPI were resolved using the following modification of gel and electrode buffer system 8 of Soltis et al. (1983): electrode buffer composed of 0.039 M lithium hydroxide, 0.263 M boric acid, pH 8.0; gel buffer composed of 0.033 M tris, 0.005 M citric acid, 0.004 M lithium hydroxide, 0.030 M boric acid, pH 8.0. Gel and electrode buffer system 9 was used to resolve PGM and MDH. IDH, [NAD]G3PDH, and 6-PGD were resolved on system 1. All enzymes except ADH and LAP were stained following Soltis et al. (1983); ADH and LAP were stained following Soltis (1986).

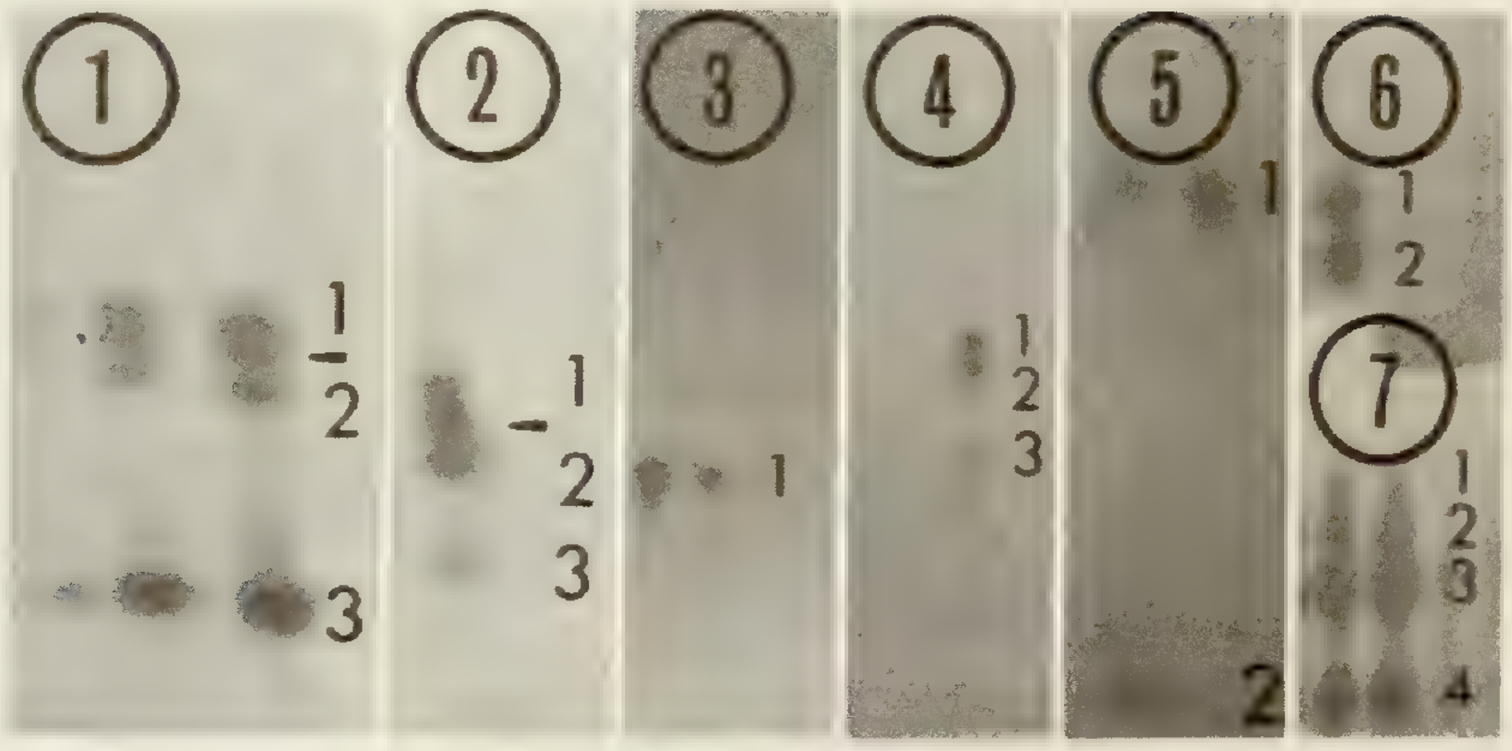
Results and Discussion

For all enzymes examined, the observed bands migrated anodally. Clear banding was observed for megaspores of *Marsilea vestita* for ADH, EST, FE, [NAD]G3PDH, IDH, MDH, ME, PGI, PGM, 6-PGD, and TPI. No activity was observed for SkDH; very faint activity was occasionally observed for AAT and LAP. Especially clear and intense banding was obtained for ADH, IDH, MDH, ME, PGI, 6-PGD, and TPI. The oldest sporocarp of *M. vestita* from which active enzymes were obtained was taken from a 32-year-old herbarium specimen.

Only a small amount of sporocarp material of *Regnellidium* was available; hence, it was not assayed for all enzymes. Clear banding was achieved for ADH, ME, PGI, and TPI. The sporocarp of *Regnellidium* analyzed was taken from a 30-year-old specimen.

Marsilea vesitata was investigated most intensively and results for each enzyme follow (Figs. 1–7). For both sporophyte tissue and megaspores, similar banding patterns were observed. For ADH, three bands were expressed: two darkly staining, closely associated bands, and a more slowly migrating fainter band (Fig. 4). For EST and FE, two and three bands, respectively, were observed. Two bands were observed for each of the following enzymes of glycolysis and the pentose-phosphate pathway: [NAD]G3PDH, PGI (Fig. 5), PGM (Fig. 6), and 6-PGD. Four bands were consistently observed for MDH (Fig. 7). For TPI, a closely associated group of three bands, as well as a more slowly migrating band, were detected (Figs. 1, 2). A single band was observed for both IDH and ME (Fig. 3).

The major purpose of this investigation is to document that active enzymes can be derived from sporocarps (some of which may be as old as 32 years). It is also interesting to note, however, that the results for most enzymes are consistent with the number of loci expected in diploid seed plants (Gottlieb, 1981, 1982). It is now well established that, despite high chromosome numbers, ferns are isozymically diploid (see Haufler and Soltis, 1986, for review). The chromosome numbers reported for *Regnellidium diphyllum* ($2n = 38$) and species of *Marsilea* ($n = 20$) are low, however, compared to most homosporous ferns (Löve et al., 1977; Tryon & Tryon, 1982). For PGI, PGM, 6-PGD, and [NAD]G3PDH, all en-



FIGS. 1–7. Photographs of starch gels showing enzyme banding from megaspores of *Marsilea vestita* and *Regnellidium diphyllum*. Putative loci are numbered sequentially with the most anodally migrating locus numbered 1. FIG. 1. *Regnellidium diphyllum*. FIGS. 2–7. *Marsilea vestita*. FIGS. 1–2. TPI, arrows between loci 1 and 2 indicate presumed interlocus heterodimer. FIG. 3. ME. FIG. 4. ADH. FIG. 5. PGI. FIG. 6. PGM. FIG. 7. MDH.

zymes of glycolysis or the pentose-phosphate pathway, two isozymes were evident in *Regnellidium* and *Marsilea*. Two isozymes are also observed for these enzymes in diploid angiosperms and gymnosperms (Gottlieb, 1981, 1982). For ADH and MDH, three and four bands, respectively, were observed in both sporophytes and megaspores. These observations are also consistent with the number of isozymes typically observed for these enzymes in diploid seed plants (Gottlieb, 1981, 1982). The only exception to diploid isozyme expression is TPI, which appears to have three, rather than two isozymes (the number typical of diploid seed plants) in both *Marsilea vestita* and *Regnellidium diphyllum*. Similar results for TPI have also been reported for most other ferns (Gastony & Gottlieb, 1985; Haufler, 1985; Haufler & Soltis, 1986). However, some ferns, such as species of *Botrychium*, do have the expected diploid number of two isozymes for TPI (Soltis & Soltis, 1986).

The observation that active enzymes can be obtained from megaspores of Marsileaceae is comparable to the results of Gastony and Gottlieb (1982) and Haufler and Soltis (1984) who found that it is possible to obtain enzyme banding from individual gametophytes of homosporous ferns. These findings indicate that genetic analyses of Marsileaceae, as well as other heterosporous pteridophytes, such as *Selaginella* and *Isoetes*, can easily be conducted for heteromeric enzymes by isolating megaspores from individual sporocarps and examining these pooled megaspores using standard electrophoretic procedures.

Active enzymes were obtained from a 30-year-old sporocarp of *Regnellidium diphyllum* and from sporocarps of *Marsilea vestita* as old as 32 years. That active enzymes can be obtained from sporocarps of such age indicates that the Marsileaceae offers unusual opportunities for systematic study. Herbarium specimens could provide a source of "living" material for electrophoretic investigation. For example, herbarium specimens could be used to increase the number of populations surveyed. In addition, herbarium specimens could serve as an important source of rare taxa, or taxa that are difficult to obtain (such as *Regnellidium*). The Marsileaceae also affords unusual opportunities from a popu-

lation biology perspective. It might be possible, for example, to analyze temporal variation in allele frequencies in a single population. This would necessitate the availability of numerous collections from a single site spanning many years.

ACKNOWLEDGMENT

We thank Dr. Amy Jean Gilmartin for the use of herbarium specimens.

LITERATURE CITED

ANDERSON, O. M. and D. O. ØVSTEDAL. 1983. Anthocyanin content in *Saxifraga svalbardensis* and some allied species. Biochem. Syst. Ecol. 11:239–241.

GASTONY, G. J. and L. D. GOTTLIEB. 1982. Evidence for genetic heterozygosity in a homosporous fern. Amer. J. Bot. 69:634–637.

——— and ———. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–267.

GIANNASI, D. E. and K. J. NIKLAS. 1981. Comparative paleobiochemistry of some fossil and extant Fagaceae. Amer. J. Bot. 68:762–770.

GOTTLIEB, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7:1–46.

———. 1982. Conservation and duplication of isozymes in plants. Science 216:373–380.

HAUFLER, C. H. 1985. Enzyme variability and modes of evolution in the fern genus *Bommeria*. Syst. Bot. 10:92–104.

——— and D. E. SOLTIS. 1984. Obligate outcrossing in a homosporous fern: Field confirmation of a laboratory prediction. Amer. J. Bot. 71:878–881.

——— and ———. 1986. Genetic evidence suggests that homosporous ferns with high chromosome numbers are diploid. Proc. Natl. Acad. Sci.: in press.

JOHNSON, D. M. 1985. New records for longevity of *Marsilea* sporocarps. Amer. Fern J. 75:30–31.

LÖVE, A., D. LÖVE, and R. E. G. PICHI SERMOLLI. 1977. *Cytotaxonomical atlas of the Pteridophyta*. Vaduz: J. Cramar.

SOLTIS, D. E. 1986. Isozyme number and enzyme compartmentalization in *Equisetum*. Amer. J. Bot.: in press.

——— and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). Amer. J. Bot.: in press.

———, C. H. HAUFLER, D. C. DARROW, and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.

American Fern Journal 76(1):21–24 (1986)

Germinating Spores and Growing Sporelings of Aquatic *Isoëtes*

W. Carl Taylor and Neil T. Luebke
Botany Section, Milwaukee Public Museum, Milwaukee, WI 53233

Several techniques have been described recently for germinating spores of aquatic species of *Isoëtes* which grow in lakes, ponds, and streams in eastern North America. Boom (1980) used glass vials containing sterile sand and pond water in which he successfully raised sporelings of *I. engelmannii, I. flaccida, I. macrospora,* and *I. riparia.* Kott and Britton (1982) germinated spores of *I. acadiensis, I. echinospora, I. macrospora, I. riparia,* and *I. tuckermanii* in small vials half filled with distilled water. However, many of their vials became contaminated with fungi that destroyed the viability of the megaspores. Sam (1982) germinated megaspores of *I. engelmannii* in an inorganic nutrient medium supplemented with Nystatin to inhibit fungi. Webster (1979) described a technique for germinating spores of *Selaginella* that is similar to the following procedure we use for *Isoëtes.*

We have been culturing plants from spores of *Isoëtes acadiensis, I. echinospora, I. engelmannii, I. hieroglyphica, I. macrospora, I. riparia,* and *I. tuckermanii* for use in germination and hybridization experiments for several years. The aquatic species of *Isoëtes* are ideal pteridophytes for germination and hybridization experiments. Their sporangia are large and contain many spores. Megaspores and microspores are borne in different sporangia on separate leaves. If necessary, unopened sporangia can be excised from sporophylls intact and surface cleansed to eliminate extraneous spore contamination. Thus, megaspores and microspores can be completely isolated for controlled breeding experiments. Also, their spores readily germinate in demineralized water and gametophytes and sporophytes develop normally for several months without supplemental nutrients.

Spores are obtained from plants collected in September and October, since plants harvested earlier in the year may not have mature, viable spores. *Isoëtes hieroglyphica* and *I. macrospora* retain their spores through the winter, so mature spores of these species can also be gathered in the spring.

Megaspores are cleansed of microspores in a sieving apparatus made of plastic tubing approximately 10 cm long and 2.5 cm in diameter. An 8 cm square of 0.27 mm sifter mesh (available from Carolina Biological Supply Company) is fitted to one end of the tubing with a rubber band. The sieving apparatus, containing megaspores, is attached to a water faucet using plastic strapping tape. Spores are washed with a steady flow of cold water for 30 minutes, followed by a one minute rinse with sterile, demineralized water.

Although megaspore surfaces appear clean after the above treatment, unopened sporangia are preferred for crossing experiments because there is less chance of contamination from microspores. Intact sporangia can be excised from

sporophylls using a sharp scalpel and fine forceps. Outer whorls of sporophylls contain the most mature spores. By cutting on the abaxial side along its point of attachment to the corm, a sporophyll can be removed without rupturing the sporangium. Leaf tissue is carefully peeled away from the sporangium using forceps (Fig. 1). Excised sporangia are cleaned with a sieving apparatus in the manner described above for megaspores. Sporangial walls of *I. riparia*, *I. macrospora*, and *I. hieroglyphica* are relatively strong, making their sporangia easy to remove without rupture. Sporangial walls of *I. echinospora*, *I. engelmannii*, *I. tuckermanii*, and *I. acadiensis* weaken rapidly when their spores are mature. This makes extraction of intact sporangia more difficult. If unopened sporangia of these species are desired, plants should be collected and their sporangia extracted by mid-September. Plants of all aquatic species may be kept for a week or more by rinsing, draining, and sealing the plants in plastic bags which are kept cool until the sporangia are removed.

Using aseptic technique, spores or sporangia are transferred with forceps to plastic tissue culture dishes. We use 35 mm × 10 mm size dishes (Corning 25000) each containing 4 ml of sterile, demineralized water. Intact megasporangia are opened and their contents dispersed. Generally, 30 to 60 or more megaspores are put in each dish. Microspores may be added to the cultures by briefly swirling part of a ruptured microsporangium in each dish. Dishes are sealed with 12 mm × 100 mm strips of parafilm to prevent evaporation of the water.

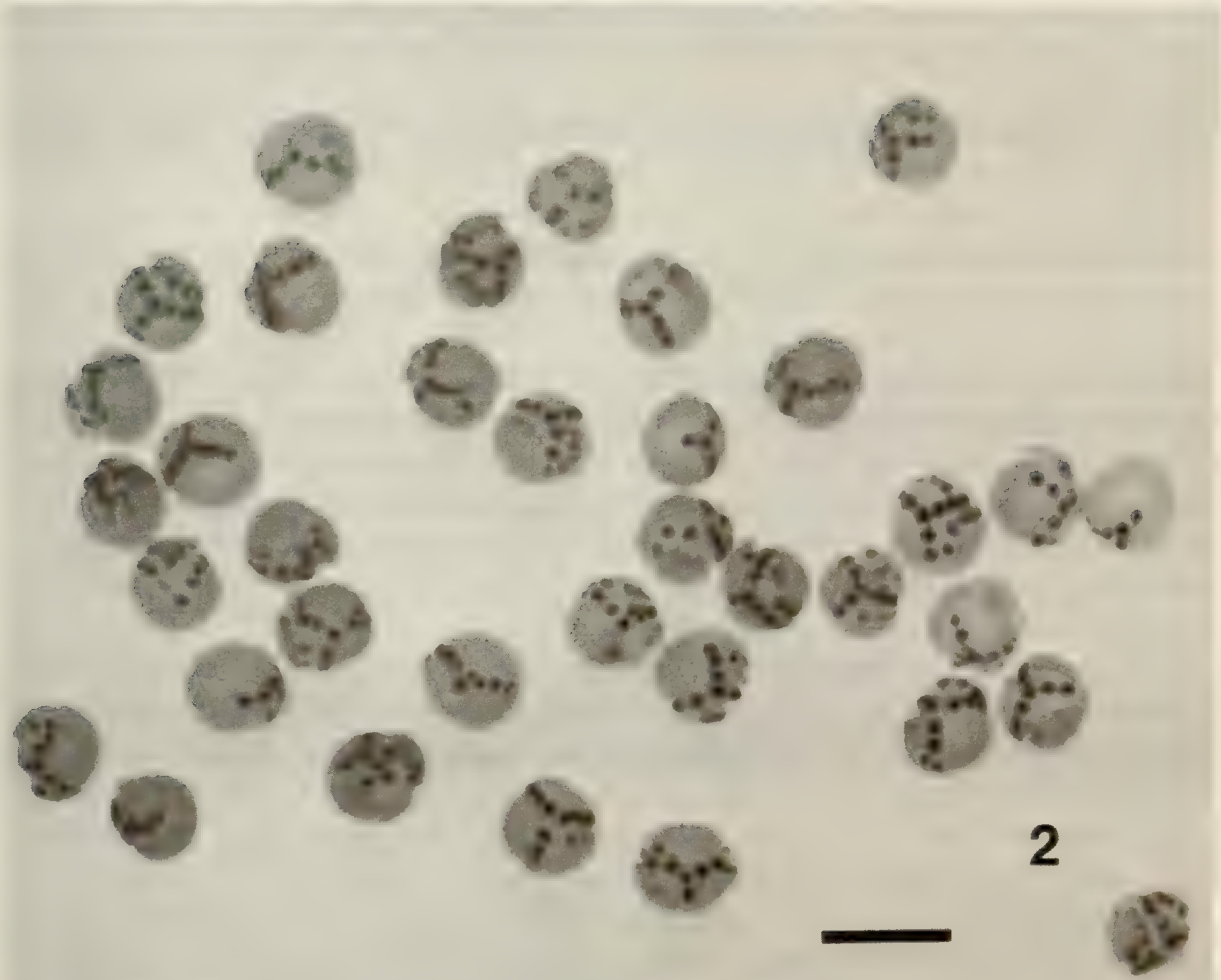
Cultures are vernalized in the dark for 100 days at 2°C prior to their incubation at 20°C with a 12L:12D photoperiod using 20 watt fluorescent grow lights 25 cm above the cultures. Experiments indicate that only *I. echinospora* requires this vernalization. Although spores of the other species germinate without cold treatment, vernalization accelerates germination and sporophyte formation.

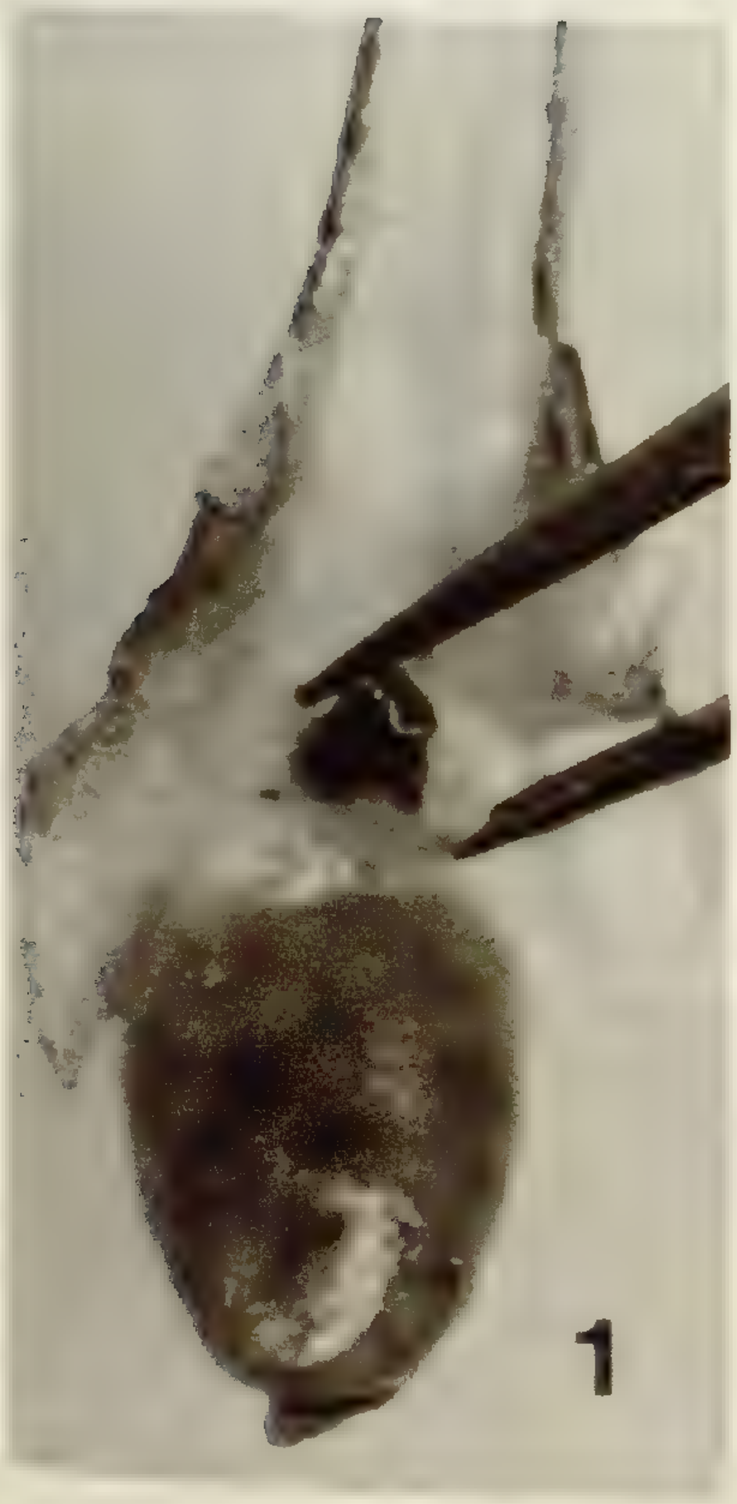
When the above procedure is followed, the first megagametophytes can be expected within 10 days after the start of incubation. A few days later, archegonia can be recognized by brown pigmented quartets of neck cells on the megagametophytes (Fig. 2). Cultures containing both megaspores and microspores normally develop sporophytes within 10 to 30 days depending on the species (Fig. 3). In our cultures, *I. echinospora*, *I. engelmannii*, and *I. riparia* usually form gametophytes and sporophytes sooner than *I. acadiensis*, *I. hieroglyphica*, *I. macrospora*, and *I. tuckermanii*.

Initially, we used the fungicide Nystatin in our cultures at 100,000 units/liter of sterile, demineralized water, but we have found it unnecessary in subsequent experiments. Using the above procedure we have had little problem with fungal contamination. It is also unnecessary to add nutrients to the cultures. Indeed, the addition of nutrients can increase the growth of any contaminating organisms. Megaspores of *Isoëtes* contain sufficient food reserves to support devel-

→

FIGS. 1–3. Cultural techniques in *Isoëtes*. FIG. 1. Excising megasporangium from sporophyll. FIG. 2. Pure culture of *Isoëtes macrospora* megagametophytes approximately 120 days old; bar = 1 mm. FIG. 3. Sporophytes, approximately 90 days old.


2


1

3

oping gametophytes and sporophytes for several months. By that time sporophytes are old enough to be transferred to a sand, gravel, and peat substrate in larger dishes.

We have had the most success when a young sporophyte is transferred shortly after the emergence of its first root. At this stage we move the sporophytes to glass storage dishes (Corning 3250) containing a 2.5 cm layer of substrate. The substrate is a mixture of 4 parts fine sphagnum peat, 3 parts fine quartz sand, and 3 parts medium quartz sand. Sporophytes are placed on top of this mixture and covered with a thin layer of medium quartz sand. The additional layer of sand anchors young plants to the substrate and promotes rooting. This is important for continued sporophyte development because floating plants slowly deteriorate. Demineralized water is added to a level approximately 1 cm above the sand. After water is added and any floating plants anchored, each dish is covered with a glass lid to reduce evaporation. The young sporophytes are cultured at 18°C with a 15L:9D photoperiod using 20 watt fluorescent grow lights 20 cm above the dishes.

As the plants continue to grow water depth is gradually increased. Within a year the sporophytes are transferred to 2½″ (6.4 cm) square plastic pots containing the substrate described above. This substrate is covered with a thin layer of quartz gravel to keep the peat from floating as the potted plants are submerged in 20 gallon aquaria. Nutrients for plant growth are provided by several small fish living in each aquarium. May through October, the aquaria are maintained at approximately 18°C with a 15L:9D photoperiod using four 30 watt fluorescent lights above each tank. December through March, the aquaria are kept at 2°C and each day receive 8 hours of very low light from two fluorescent ceiling lights. April and November are transition months during which the temperature and daylength are gradually adjusted to the above conditions. Sporophytes of aquatic *Isoëtes*, collected from their native habitats, are grown under the same conditions.

The methods described in this paper for germinating spores and growing sporelings of *Isoëtes* are simple. Using these procedures, large numbers of gametophytes, and sporophytes of selected parentage, are easily produced. Germination studies and crossing experiments can be set up without contamination from unwanted spores and resulting sporophytes can be grown for further study.

Literature Cited

Boom, B. M. 1980. Intersectional hybrids in *Isoëtes*. Amer. Fern J. 70:1–4.

Kott, L. and D. M. Britton. 1982. A comparative study of spore germination of some *Isoetes* species of northeastern North America. Canad. J. Bot. 60:1679–1687.

Sam, S. J. 1982. A germination method for *Isoëtes*. Amer. Fern J. 72:61.

Webster, T. R. 1979. An artificial crossing technique for *Selaginella*. Amer. Fern J. 69:9–13.

Shorter Notes

***Pellaea brachyptera* New to Washington.**—On 9 July 1984, the Sierra cliff brake, *Pellaea brachyptera* (Moore) Baker, was encountered while conducting a botanical survey of the Lake Chelan-Sawtooth Wilderness for the Washington Native Plant Society. Vouchers were collected (*Alverson 799*, MICH, ORE, UC, US, WS, WTU) from a large colony above the Lakeshore Trail #1247, 1 km north of Prince Creek, in the Wenatchee National Forest of Chelan County. The Washington populations are restricted to a small area along the upper reaches of Lake Chelan, an 88 km long fiord-like body of water that occupies a glacial trough extending from the Cascade Range on the northwest to the arid Columbia Basin on the southeast. This cliff brake was previously known to range only from northern California (Placer and Lake counties) north to southwestern Oregon (Lane County) (Munz, *A California flora and supplement*, 1973; D. H. Wagner, pers. comm.). The Chelan County populations represent a northerly outlier some 500 km disjunct from the nearest population in southwestern Oregon. Thus, *P. brachyptera* is a new addition to the flora of Washington, as well as to the Pacific Northwest flora as delineated by Hitchcock and Cronquist (*Flora of the Pacific Northwest*, 1973).

Actually, *P. brachyptera* was first collected in Washington by Ralph and Dorothy Naas in 1971, along the Prince Creek trail about one mile up from Lake Chelan. The specimen, #938b (deposited in the North Cascades National Park herbarium), was misidentified as *Pellaea glabella* var. *simplex*, a species found on shaded basalt cliffs in the nearby Columbia Basin.

We observed the greatest concentration of *P. brachyptera* plants on south- to west-facing slopes between Prince Creek and Rattlesnake Creek, at elevations of 330 to 600 m, in the south half of Section 29, Township 31 N, Range 19 E. Total number of plants in this population, spread over at least one hundred hectares, probably exceeds one thousand. Further surveys along the Lakeshore Trail revealed the presence of four smaller populations, extending as far uplake as Round Mountain, about 11 km northwest of Prince Creek. Field sighting forms giving the precise locations of these additional populations have been filed with the Washington Natural Heritage Program in Olympia, WA, which has added *P. brachyptera* to the list of sensitive plant species in Washington.

Along Lake Chelan, *Pellaea brachyptera* grows on open, dry, south- to west-facing slopes that support sparse stands of *Pinus ponderosa* and *Pseudotsuga menziesii*. Associates include *Purshia tridentata*, *Agropyron spicatum*, *Bromus tectorum*, *Balsamorhiza sagittata*, *Achillaea millefolium*, *Hieracium albertinum*, and *Collomia grandiflora*. *Pellaea brachyptera* grows in rocky soil and around the base of boulders, a habitat shared with the equally abundant *Aspidotis densa*. The soils are derived from the regional bedrock, Swakane Biotite Gneiss, as well as glacial deposits such as till and unsorted outwash (Cater & Wright, U.S.G.S. geological map, 1967). The exposed slopes serve to accentuate the effects upon the vegetation of the regional climate, which is characterized by hot, dry summers and cool, snowy winters. Individual plants of *P. brachyptera* are often

quite robust, in some cases forming clumps up to 1 m in diameter, with hundreds of fronds.

The habitat has been degraded as a result of many decades of grazing by sheep and horses, and the possibility of this population having originated as an accidental (or even deliberate) introduction must be considered. However, the likelihood of this having happened is slight. The Washington populations appear to be both too remote, and too well established, to have originated within the last century. A more plausible interpretation is that they are remnants of a more continuous distribution along the eastern base of the Cascade Range.

Interestingly, several flowering plants exhibit a pattern of disjunction similar to that of *P. brachyptera*. *Arctostaphylos patula* (Ericaceae), *Spiranthes porrifolia* (Orchidaceae), *Pectocarya pusilla* (Boraginaceae), and *Githopsis specularioides* (Campanulaceae) are among plants of southern distribution that occur in disjunct populations along the upper reaches of Lake Chelan. In general, plants of southern affinities migrated northward and reached their northern range limits during the hypsithermal interval that followed the close of the last glacial period (Detling, Bull. Mus. Nat. Hist. Univ. Oregon 3:1–57, 1968). Most probably, *Pellaea*, *Arctostaphylos*, *Spiranthes*, *Pectocarya*, and *Githopsis* extended their ranges northward to Lake Chelan during this warmer period. As the climate subsequently cooled to present conditions, these plants were able to persist locally along Lake Chelan, while connecting populations to the south were extirpated. The great diversity of habitats and vegetation along Lake Chelan is an indication that the area is particularly suitable for the retention of relicts. The influence of Lake Chelan in moderating cold winter temperatures is likely to have been a factor as well.

Thus, the Chelan County populations of *Pellaea brachyptera* are interpreted here as a northern outlier of a once more or less continuous series of populations. This is in contrast to previously documented fern disjunctions, such as *Polystichum scopulinum* (Wagner & Rouleau, Amer. Fern J. 74:33–36, 1984), and *Pellaea wrightiana* (Wagner, J. Elisha Mitchell Sci. Soc. 81:95–103, 1965), in which long distance spore dispersal is suggested as the most probable means of origin. However, it should be noted that these disjunctions follow the prevailing (west to east) direction of upper atmosphere air movement. The Washington populations of *Pellaea brachyptera*, in contrast, are located north of the species primary range, and south to north atmospheric air currents are relatively uncommon.

The authors thank Ron Taylor and Joe Miller of the Washington Native Plant Society for originating and supporting the Chelan-Sawtooth field survey, and Ralph and Dorothy Naas for providing information on their original sighting of *Pellaea brachyptera*.—EDWARD R. ALVERSON, 12530 SE 47th Pl., Bellevue, WA 98006, and JOSEPH ARNETT, Biology Dept., Western Washington University, Bellingham, WA 98225.

A Second Eastern North American Occurrence for Forked Spleenwort, *Asplenium septentrionale*.—An unusually interesting group of North American ferns are those that have their main occurrence in western North America with

one or a few outliers hundreds of kilometers to the east (Wagner, W. H., Jr., Ann. Missouri Bot. Gard. 59:203–207, 1972). They include such species as *Cheilanthes castanea, Pellaea wrightiana, Aspidotis densa, Polystichum scopulinum* and the forked spleenwort, *Asplenium septentrionale* (L.) Hoffm. The last of these was considered until recently to be entirely a western species. Its easternmost localities were in southwestern South Dakota, Colorado, western Oklahoma, and Texas (Lellinger, D. B., *A field manual of the ferns and fern allies of the United States and Canada*. Smithsonian Inst. Press, 1985), until this unusual spleenwort was discovered far to the east near Waiteville, Monroe County, West Virginia (Emory, D. L., Amer. Fern J. 60:129–134, 1970).

On 9 June 1985, while botanizing along an unpaved road in Hardy County, West Virginia, I encountered a small stand of plants that I thought from a distance to be a tall cluster of mosses. Looking closer on the rocky ledge, I recognized immediately that I had discovered an additional population of *Asplenium septentrionale* for West Virginia. This find represents the second stand some 2000 kilometers from the nearest known station in western Oklahoma and is approximately 225 kilometers northeast of the Monroe County locality.

At the time of this discovery the Brooks Bird Club was conducting a foray in Hardy County, so that several members were able to substantiate my identification. Later Rodney Bartgis, William Grafton, and Robert Richardson checked the surrounding territory within a radius of 45 meters from my first colony of the forked spleenwort, and they located 120 living plants, but all of the rock outcrops were not explored and later additional plants were found in September. Twenty dead plants were also noted. My second trip to the locality was on 29 September, when I was accompanied by W. H. Wagner, Jr., F. S. Wagner, and Kyle Bush. We not only explored more outcrops but we also recorded additional associated plants.

The topography of the Hardy County site varies from sandstone rock outcrops to loose crumbling shale that predominates the area. Rocks are of the Hampshire formation, a series of red non-Devonian shales and sandstones (Cardwell, D. H., *Geologic history of West Virginia*. Geological and economic survey, 1975, p. 64). Unlike the plants at the Monroe County locality, which grow on loose chips of shale (Emory, 1970, fig. 1), ours grow on much firmer rock, usually in horizontal cracks in otherwise continuous, unbroken rock surfaces. The forked spleenwort population occurs at an elevation of 730 to 850 meters. The rock outcrops face southward and the plants tolerate various exposures from 0 percent where the rock ledges are at the edge of the woods to 20–70 percent in the woods.

In order to define the habitat as well as possible, the following list of associated species was prepared (those marked with asterisks are especially prominent). Woody plants include *Amelanchier arborea, Crataegus uniflora, Parthenocissus quinquefolia, Pinus pungens, *P. virginiana, P. strobus, *Quercus coccinea, *Q. ilicifolia, *Q. prinoides, Q. rubra, Q. velutina, Robinia pseudoacacia, *Rubus allegheniensis, Spiraea corymbosa,* and *Vaccinium stamineum*. The forbs include *Allium cernuum, Antennaria virginica, Aster ericoides, Cynoglossum officinale, *Danthonia* sp., *Draba ramosissima, Eupatorium rugosum, Fragaria virginiana, Heuchera* sp., *Hieracium trailii, Houstonia longifolia, Penstemon*

laevigatus, *Phlox subulata*, *Saxifraga virginiensis*, *Silene pensylvanica*, *Sisymbrium altissimum*, and *Solidago caesia*.

The plants of forked spleenwort at this locality are not only much more numerous but are much larger than those at the Monroe County locality. Fronds reach to over 12 cm and the largest tufts measure 10 cm in diameter with hundreds of fronds. Practically all of the fronds are forked only once and only near the tip in the apical 1 cm in contrast to the form of the species that forks several times and much lower on the rachis (e.g., Page, C. N., *The ferns of Britain and Ireland*. Cambridge Univ. Press, 1982, fig. 13). Other ferns found on the rock outcrops are much less common. *Asplenium platyneuron* is the most numerous, but most plants of that grow on soil; only a few are found upon the rock surfaces. *Asplenium montana*, *A. trichomanes*, *Dennstaedtia punctilobula*, *Polypodium virginianum*, and *Woodsia obtusa* are all found on the same outcrops as *A. septentrionale*, but except for *Woodsia* are very rare in the area, and all of them are more or less dwarfed. A hybrid involving *A. septentrionale* with possibly *A. platyneuron*, *A. trichomanes*, or *A. montana* was encountered first by Rodney Bartgis and later by Florence Wagner. It is currently being analyzed and will be reported in the near future.

Emory (1970) noted that it is entirely possible that other stations of this rare fern would be discovered, as there are numerous similar habitats. His prediction has now come true. It is also possible that other stations will be discovered between the Monroe County and the Hardy County stations and beyond. The list of associates given here should help in recognizing potential habitats. At the same time I emphasize Emory's statement regarding protection of this plant. I urge the greatest caution in maintaining these plants in their natural state without disturbance or vandalism. Vouchers and exact locality data are on record in West Virginia University Herbarium, Morgantown, WV.—ELEANOR M. BUSH, 5 Bush Avenue, Philippi, West Virginia.

Second Locality for *Dryopteris carthusiana* in Arkansas.—The spinulose wood fern, *Dryopteris carthusiana* (Villars) H. P. Fuchs [*D. spinulosa* (O. F. Muell.) Watt], was first reported for Arkansas by Lesquereux (A catalog of the plants of Arkansas. Pp. 346–399 in Owen, *Second report of a geological reconnaissance of the middle and southern counties of Arkansas made during the years 1859 and 1860*, 1860) from "woods." Based on this report, it was included in state lists by Harvey (Bot. Gaz., Crawfordsville 6:188–190, 1881) and Branner and Coville (A list of the plants of Arkansas. Pp. 155–242, in Branner, *Annual report of the geological survey of Arkansas for 1888*, Vol. IV, 1891). Buchholz (Amer. Fern J. 14:33–38, 1924) expressed doubt about the presence of this species in Arkansas, because he could not locate voucher material. Based on a discovery by Dwight M. Moore in 1924, Buchholz and Palmer (Trans. Acad. Sci. St. Louis 25:91–155, 1926) reported this species from the north side of Mt. Magazine, Logan Co., Arkansas. Recent efforts to locate a voucher or plants at that locality were also unsuccessful (Taylor & Demaree, Rhodora 81:503–548, 1979; Taylor, *Arkansas ferns and fern allies*, 1984, p. 106). The only verifiable population of *D. carthu-*

siana in Arkansas was discovered by D. M. Moore on 7 August 1960, in Stone Co., Arkansas, where approximately a dozen plants occur at the entrance of Rowland Cave. This population represents the most extreme southwestern population of this species in eastern North America (Carlson & Wagner, Contr. Univ. Michigan Herb. 15:141–162, 1982).

On 5 October 1985, while surveying the status of *Woodsia scopulina* D. C. Eaton on Mt. Magazine, I located 4 plants of *D. carthusiana* on the northside of the mountain, near its summit, in the vicinity of Brown's Spring. This population, associated with three other fern species also occurring as peripheral populations [*Dennstaedtia punctilobula* (Michx.) Moore, *Dryopteris marginalis* (L.) Gray, and *Woodsia scopulina* D. C. Eaton], is most probably the population initially discovered by Moore in 1924. Verification of the occurrence of a Mt. Magazine population extends the known range of *D. carthusiana* 300 km to the southwest of the Stone Co. population. The occurrence of this northern species in Arkansas appears to be related to "northern" environmental factors provided by elevation (860 m) at the top of the tallest mountain in Arkansas and by moderated, cool, moist air blowing from a cave entrance. Based on the known locations of *D. carthusiana* in Arkansas, it is most improbable that Lesquereau ever saw this species in Arkansas during his travels; the earlier attributions of this species in the Arkansas flora must be considered spurious.

This research was sponsored, in part, by the State of Arkansas' Nongame Preservation Program Committee, by a faculty research grant from Office of Research and Sponsored Programs, and by the College of Science, Office of Research, Science and Technology, at the University of Arkansas at Little Rock.—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

REVIEWS

"*A field manual of the ferns & fern-allies of the United States and Canada*," by David B. Lellinger with photographs by A. Murray Evans. 1985. ix + 389 pp. + 45 pp. of color photographs. Washington, D.C.: Smithsonian Institution Press. ISBN 0-87474-602-7, $45.00 (hardcover). ISBN 0-87474-603-5, $29.95 (paperback).

This manual, containing keys, descriptions, and photographs, provides a means to identify the ferns and fern-allies of the contiguous United States and Canada. Four hundred and six native and naturalized species, subspecies and varieties are treated. Three hundred and forty-one of these are illustrated by color photographs.

The well written introduction includes valuable information on collection, classification, distribution, ecology, structure, life history, and culture of pteridophytes. Sections on classification and paleontology are particularly good. Statements on culture are useful to those who grow ferns and fern-allies.

Keys and descriptions constitute most of the text. A key to families directs the user to family descriptions which are followed by keys to genera. These keys guide one to a generic description and a key to the species of that genus. For each species there is a scientific and common name, a description of diagnostic characters, and statements about habitat, abundance, distribution, and culture. Species are numbered uniformly in the keys, descriptions, and photographs to make comparisons easy. Following the species descriptions, there is a unique section titled "Hybrid Complexes" where the author graphically presents results from biosystematic studies of species complexes in the form of hypothetical pedigrees. Systematists will find this part particularly interesting.

The color photographs in this work are impressive not only for their quality but for their number. Anyone who has attempted to photograph ferns and fern-allies can appreciate the effort by Dr. Evans. Users will find these pictures helpful in confirming their determinations. The book concludes with an excellent, illustrated glossary, an extensive bibliography, a checklist of species, lesser taxa, and hybrids, and separate indices to common and scientific names.

The keys, constructed of well chosen, contrasting characters, are a pleasure to use. In addition, keys are numbered to facilitate backtracking if the wrong statement of a couplet is chosen. With this book and a minimum of experience, nearly anyone can successfully identify a pteridophyte. Drs. Lellinger and Evans have combined their talents to produce a scholarly, informative, and very usable textbook on ferns and fern-allies that certainly should be in the library of every professional and amateur pteridologist. Even those who do not yet know and love ferns could be enticed to our ranks by this fine volume.—W. Carl Taylor, Botany Section, Milwaukee Public Museum, Milwaukee, WI 53233.

"*Biology of pteridophytes*," edited by A. F. Dyer and C. N. Page. 1985. 474 pp. Proc. Royal Soc. Edinburgh, Section B, Volume 86. Edinburgh: The Royal Society of Edinburgh. ISSN-0308-2113.

This volume presents the proceedings of an international symposium held at the University of Edinburgh in September, 1983. The purpose of the symposium was to consider pteridophytes as living organisms as demonstrated both through field-oriented and laboratory studies and to maintain the momentum of the current interest in pteridophyte biology. Contributions cover an extremely wide range of topics from cell and molecular biology, genetics, water relations, and development, through ecology and diversification, modes of speciation, life-history studies, conservation, and the biology of economic ferns. There are 57 papers and 37 abstracts of poster demonstrations by 75 authors from 22 countries. Almost all of the contributions are short and leave the reader with a great desire for more complete information. However, they represent the papers given and reflect the current state of pteridophyte research as of 1983, and many contain a wealth of literature citations.

Pteridophytes in the broad sense were the first colonizers of land and today they are the second most diverse group of vascular plants after the angiosperms.

An important fact emerging from current research is the realization that pteridophytes exhibit many basic processes which, with more complete understanding, will provide phylogenetic meaning for features found in higher vascular plants, and that the key to understanding the life-cycle of seed plants lies in understanding facets of the life-cycle of the modern day remnants of their distant ancestors. For example, gametophyte/sporophyte antagonism, perhaps induced by their interaction, may represent the initial process leading to incompatibility in flowering plants. The fixation and retention of mutational load, promoted by the apical meristem of leptosporangiate ferns, may relate to selection for larger and more complex meristems in other plants providing increased buffering against disadvantageous mutations, and, indeed, even to outbreeding mechanisms. Antheridiogens are a part of this process and it is suggested that products of metabolism in ferns have become phytohormones in which a gibberellin-like substance acts as a signal for morphogenesis and that these substances are the ancestors of the gibberellin hormones which influence seed plant development. The co-evolution of insects and plants, which has culminated in the pollination relationships of flowering plants, may have had its earliest beginnings with spore-eating and transport by Devonian and Carboniferous arthropods. Chemical components and biochemical pathways of spore germination are similar to those observed in seeds and represent evolutionary experiments that took place during the evolution of the seed.

There are a number of other fascinating studies detailed in these proceedings which stimulate hypotheses on the phylogeny and evolutionary success of pteridophytes. Tannins and phenols are common in ferns. Ferns apparently have a much more limited array of anti-herbivore and anti-microbial chemicals compared to angiosperms, although many classes do have such activity. To date, however, there is only negative evidence in terms of a direct chemical response to herbivory, and insects seem to be responding to ferns rather than ferns to insects. The multi-layered structure of the male gamete bears remarkable resemblance to a flagellar root structure found in the motile phase of *Coleochaete*-like algae. Pteridophytes, bryophytes, gymnosperms, and angiosperms all have similar changes in cytoplasmic organelles and cytoplasmic ribosomes during the gametophyte-sporophyte transition. However, within pteridophytes, the variation and interaction is much greater than in any other plant group. Comparative sperm morphology reveals similarities between *Selaginella*, bryophytes, and *Marsilea*, which are profoundly different from the similarities shown by *Lycopodium*, *Equisetum*, *Ginkgo*, homosporous ferns, and cycads. This suggests that *Lycopodium* and *Selaginella* are phyletically distantly related and that homosporous and heterosporous ferns are unrelated.

Recent studies in physiology and water relations are providing new clues by which we may gain a greater understanding of pteridophyte ecology. Ability to withstand vegetative desiccation is more common in pteridophytes than in seed plants in both the sporophyte and gametophyte generations. This is a feature shared with bryophytes and algae. In addition, sporophytes and gametophytes are genotypically shade-tolerant and can grow at very low photon flux densities (a feature that might explain the abundance of fern spores immediately above

the Iridium layer at the Cretaceous/Tertiary boundary). In addition, photosynthesis of sporophytes is not saturated by normal atmospheric carbon dioxide levels, even at low oxygen tension, a feature perhaps adaptive at the time of the initial radiation of land plants. Although their rates of efficiency suggest that pteridophytes are capable of growing under stress, their low potential growth rate results in a general inability to become effective annual plants. Thus, they are not often capable of exploiting frequently disturbed habitats.

Other studies suggest that hybrids are common in *Isoëtes*, *Equisetum*, *Botrychium*, and *Lycopodium*, and that outbreeding in taxa with underground gametophytes must be more prevalent than commonly believed. Pteridophyte hybrids in the tropics occur most frequently in disturbed habitats but their relative frequency appears to be considerably less than in temperate regions.

Two new approaches in fern research show great promise for clarifying many of our current controversies in systematics and evolutionary biology. These include molecular advances in chloroplast DNA hybridization and protein enzyme analysis and life-history strategies combined with basic physiology. The molecular approaches hold great promise for clarifying systematic and phyletic relationships as well as uncovering patterns of population genetic diversity. Studies of breeding systems and life-histories will do much to increase our understanding of the ecology of pteridophytes as a whole.

This review only touches upon the new research and ideas presented in this volume. Taken together, the papers indicate that current research in pteridology is important to and is of interest to the botanical public as a whole. Those of us who study this group of organisms will benefit greatly by the breadth of information contained within.—JUDITH E. SKOG, Department of Biology, George Mason University, Fairfax, VA 22030; and ROBERT M. LLOYD, Department of Botany, Ohio University, Athens, OH 45701.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

BOOKS ON FERNS

An extensive list of floras, monographs, and horticultural manuals is available for two first-class stamps. I also buy used fern books; please send list of authors, titles, and dates. Myron Kimnach, 1600 Orlando Rd, San Marino, CA 91108.

BRITISH PTERIDOLOGICAL SOCIETY

Open to all who are interested in growing and studying ferns and fern-allies. Full members receive THE FERN GAZETTE and the BULLETIN OF THE BRITISH PTERIDOLOGICAL SOCIETY. Membership subscriptions are £ 7 for full members, £ 5 for ordinary members (not receiving the GAZETTE), and £ 5 for student members (under 25 years of age). For particulars, U.S. residents should apply to Dr. David S. Barrington, Pringle Herbarium, Department of Botany, University of Vermont, Burlington, VT 05405. Non-U.S. residents should apply to Hon. Gen. Sec., % British Pteridological Society, British Museum (Natural History), Cromwell Road, London SW7 5BD, England.

AMERICAN FERN JOURNAL

Volume 76

Number 2

April–June 1986

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Three New Species of Moonworts (*Botrychium* subg. *Botrychium*) Endemic in Western North America — *W. H. Wagner, Jr.* and *F. S. Wagner* — 33

Gametophytes of *Lycopodium lucidulum* from Axenic Culture — *Dean P. Whittier* and *Terry R. Webster* — 48

Revision of the Neotropical Fern Genus *Cyclodium* — *Alan R. Smith* — 56

Shorter Notes

A New Substrate for *Ophioglossum palmatum* in Florida — *Clifton E. Nauman* and *Richard Moyroud* — 55

***Cystopteris tennesseensis* in West Virginia** — *Allison W. Cusick* — 99

Corrections to *Index Filicum* — 47

Information for Authors — Cover 3

The American Fern Society

Council for 1986

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *President*

JUDITH E. SKOG, Biology Dept., George Mason University, Fairfax, VA 22030. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

JAMES D. MONTGOMERY, Ecology III, R.D. 1, Berwick, PA 18603. *Back Issues Curator*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Science, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH . . . . . Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY . . . . . Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER . . . . . Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER . . . . . U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER . . . . . Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editor welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 76(2):33–47 (1986)

Three New Species of Moonworts (*Botrychium* subg. *Botrychium*) Endemic in Western North America

W. H. WAGNER, JR. and F. S. WAGNER
Department of Biology, The University of Michigan, Ann Arbor, MI 48109

Probably no group of modern ferns or fern-like plants is as poorly understood as the Ophioglossales. Interpretations of the "fertile segment" (sporophore) and the unusual stele (eustele?) have troubled botanists for many years. Even the relationships of the order as a whole are in doubt. At the genus and species level, there has been little agreement, and the taxonomy is still up in the air. E. P. St. John (1949) grappled with some of these problems and gave a theory to explain them, based upon the subterranean gametophyte, which he believed inhibits hybridization and outcrossing. He stated that "hybridization, or even cross-breeding within the species, is almost impossible" and that as a result, the species tend to be very widely distributed. "One of the outstanding peculiarities of this group of plants," he wrote, "is that while they are of world-wide distribution the number of species is very small." Local endemics may be geographic variants of other species. According to St. John, the species of the Ophioglossaceae are closely related, and the course of evolution is usually indicated by transitional and juvenile forms. Through selfing of the subterranean gametophytes a trivial variation may form a colony, and such divergent forms might persist so long as they meet minimum requirements for survival.

Now, over a third of a century after St. John's interesting proposals, we question a number of his points on the basis of many new data. We find no evidence that subterranean gametophytes inhibit hybridization and outcrossing. Indeed, 17 hybrids are known in *Lycopodium*, 1 in *Psilotum*, and 12 in *Botrychium* (Wagner et al., 1985) plus two additional *Botrychium* hybrids described below—all taxa with subterranean gametophytes. The idea that the species of Ophioglossales are generally widely distributed is not supported by the data; only a few have truly broad distributions, such as *Botrychium lunaria*, *B. virginianum*, and *Ophioglossum nudicaule*. Most species have narrow ranges, e.g. *B. subbifoliatum*, endemic to Hawaii, *B. lunarioides*, southeastern United States, *B. oneidense*, northeastern North America, *B. echo*, southwestern United States. The true ranges were obscured by the crude taxonomies of a third of a century ago. *Botrychium boreale* became much delimited geographically when it was found that the Pacific Northwest plant so-named belongs to another species, *B. pinnatum*. We used to believe that *B. ternatum* occurred both in Asia and in North America, but we now know that the American plant is a distinct species, *B. rugulosum*, a narrow endemic, known only in the Great Lakes region from Montreal to Minnesota (Wagner & Wagner, 1982).

It is not true that members of the Ophioglossales lack diversity. St. John mentions *Helminthostachys* and *Cheiroglossa* as distinctive genera, but what about such distinctive elements within genera as *Ophioglossum bergianum* or *O. pen-*

MISSOURI BOTAN
OCT 6 19

dulum, or *Botrychium lunarioides*, *B. lanuginosum*, and *B. paradoxum*? Even major variations, like the dissected form of *B. dissectum*, may form a welter of intermediates. The dissected and undissected forms may run together in the same habitat; there is no evidence that inbred colonies are formed. Research in Japan by Norio Sahashi of Toho University has uncovered remarkable diversity in evergreen grapeferns, *Botrychium* subg. *Sceptridium*, and research by us in western North America has revealed the same in the moonworts, *Botrychium* subg. *Botrychium*. In contrast to the six well known species of western Europe and eastern North America, the species of western North America have been poorly collected, and even now some are reported from only several localities. Because of new ample population samples, we now know that western North America is the metropolis for moonworts. Including the three to be described here, there are 13 known moonwort species now in the west, as well as a number of sterile hybrids, of which the remarkable and apparently self-reproducing *B.* × *watertonense* is the best known (Wagner et al., 1984). Still other species probably exist in the grasslands and mountains of western North America, and we urge field botanists to make a special effort to locate them.

The three species and two hybrids described below are all very rare. For all three species we have been fortunate in finding excellent localities, so that we can base our descriptions on hundreds of plants. One, the Prairie Moonwort, has been collected, until recently, mainly in Canada. The Stalked Moonwort has also been taken mainly in Canada (Saskatchewan to British Columbia); only one United States locality has as yet been discovered. The Upward-lobed Moonwort has, so far, a distinctive distribution, from Nevada to the Yukon and northern Ontario. Future field explorations will unquestionably reveal additional localities and populations. We urge collectors to search for "Botrychium Havens" such as the Wallowa Mountains of Oregon, where no fewer than 10 species and two hybrids, including those described here, are now known (cf. Mason, 1975).

A key is given below to all of the moonwort species known in western North America, an area defined as west of a line from Winnipeg, Manitoba to Dallas, Texas. There is some evidence of "leakage" of certain western disjuncts east of this line, especially along Hudson Bay and western Great Lakes (Wagner & Wagner, unpublished). (The western taxon, *B. pumicola*, has not been included in the key because its status is at present in question; according to research in progress it appears to us to be a form of the extremely variable *B. simplex*.) The key is based upon large, well developed plants of each of the species. To determine young or depauperate plants, we must use associations with large plants, luster and color, spore size (all members of this group produce spores, even when they first appear above ground), and the cutting, which is usually a simplified form of the adult cutting.

Key to *Botrychium* subg. *Botrychium* in Western North America

1. Trophophore (sterile segment) absent, replaced by a second sporophore (fertile segment); sporophore branches short, mostly less than 5 mm (except the lowest branches in very large plants) ..... *B. paradoxum*

1. Trophophore present with sporangia scattered along margins, or (usually) sporangia absent altogether; sporophore branches longer, mostly more than 5 mm.
   2. Trophophore narrow, completely ringed along the margins with sporangia; spores irregular in size and shape ..... *B. ×watertonense*
   2. Trophophore with a few sporangia at base (very rarely extra sporophores at base) or (usually) no sporangia; spores regular in size and shape.
      3. Lower pinnae linear, lanceolate, or ovate, with a central or at least basal midrib.
         4. Trophophore broadly deltate, usually subsessile; pinnae linear; sporophore divided near base into two or more major axes ... . *B. lanceolatum*
         4. Trophophore mostly oblong to ovate, subsessile to long-stalked; segments linear to oblong to spatulate; sporophores with only one major axis or, if more, the laterals arising well above the base.
            5. Trophophore stalk long, 30 (10–50) percent of trophophore length; blade outline mostly triangular-ovate; lower pinnae stubby, rhomboidal and asymmetrical; common stalk (old plants) commonly with a single narrow brown stripe below the trophophore ..... *B. pedunculosum*
            5. Trophophore stalk shorter, 5–25 percent of trophophore length; blade outline mainly oblong, or triangular by exaggeration of basal pinna pair; lower pinnae more elongate, oblong to linear; common stalk lacking a single narrow brown stripe below the trophophore.
               6. Pinnae usually well separated, linear to oblanceolate with pointed tips; basal pinnae mostly cleft into a smaller lower segment and larger upper segment; lamina shiny green in life ..... *B. echo*
               6. Pinnae usually approximate, oblong to ovate with rounded or blunt tips; basal pinnae not cleft into two segments; lamina luster various.
                  7. Pinnae with few lobes, these mainly on the basal side; lowest pinnae mostly exaggerated, ascending and commonly subclasping; pinnae broadly adnate, strongly asymmetrical; lamina dull gray-green in life ..... *B. hesperium*
                  7. Pinnae with numerous lobes, these roughly equal in number on upper and basal sides; lowest pinnae conform, mostly equal in length to those above; pinnae narrowly adnate, nearly symmetrical; lamina shiny bright green in life ..... *B. pinnatum*
3. Lower pinnae or pinnules lunulate, fan-shaped, wedge-shaped or square, with flabellate venation, a central midrib lacking.
   8. Apex of trophophore usually not deeply divided, commonly con-

cave (in life); upper pinnae or lobes tending to be somewhat irregularly fused; sporophore arising at various positions, from ground level to near the apex; trophophore sessile to long-stalked.

9. Leaves herbaceous to succulent; trophophore strongly laminate, lobed to 1–2-pinnate, oblong (in young and smaller plants) to deltate (in older and larger plants); segments rounded, asymmetrical, fan-shaped to spatulate, the outer margins entire ..... *B. simplex*
9. Leaves succulent; trophophore more or less skeletonized, usually merely shallowly lobed, linear to oblong, the segments angular, commonly square or oblong, the outer margins usually irregularly and coarsely toothed ..... *B. montanum*

8. Apex of trophophore usually deeply divided, mostly flat (in life); upper lateral pinnae and lobes regularly separated; sporophore attachment high on the common stalk; trophophore sessile to short-stalked.
   10. Pinnae lunate to broadly cuneate, the sides of the lower pinnae at a 90°–180° angle, remote to overlapping.
      11. Pinna pairs (3)5–6(8), approximate to overlapping (except in shaded sites), mostly entire or nearly so; texture leathery, color (in life) shiny dark gray-green ..... *B. lunaria*
      11. Pinna pairs (2)3–4(5), usually remote, mostly crenulate to dentate; texture thinly herbaceous, color (in life) shiny light yellow-green ..... *B. crenulatum*
   10. Pinnae cuneate to oblong, the sides of the pinnae at a 0°–90° angle; usually remote.
      12. Sporophore usually only slightly longer than the trophophore; segments linear to oblong to spatulate, the largest strongly asymmetrical and bifid; spore diameter 34–39 μm (in Hoyer's solution) ..... *B. campestre*
      12. Sporophore usually 1.3–2.0 times longer than the trophophore; segments cuneate or narrowly cuneate, the largest only moderately asymmetrical, usually not bifid; spore diameter 40–50 μm.
         13. Outer pinna margins entire or undulate, not deeply dentate; pinnae usually only slightly ascending; lamina thick, not veiny, dull gray-green ... *B. minganense*
         13. Outer pinna margins deeply and irregularly dentate or serrate; pinnae strongly ascending; lamina thin, veiny, shiny yellow-green ..... *B. ascendens*

Botrychium ascendens W. H. Wagner, sp. nov. (Figs. 1, 2).—Type: U.S.A., Oregon, Wallowa Co., S of Enterprise, ca. 1.5 mi from beginning of Hurricane Creek Trail, *Wagner 83363* (MICH).

Segmenta cuneata, valde ascendentia; textum tenuis, venis prominentibus; margines valde dentati et plus minusve lobati; chromosomatum numerus $n = 90$.

FIG. 1. *Botrychium ascendens* leaves, *Wagner 83363*. Note pinna outlines, orientation, and margins.

Plants exclusive of roots reaching to 20 cm tall, averaging 10–12 cm tall, the common stalk 5 cm (2—12) tall, sporophore one-third to one-half again as long as the trophophore; trophophore bright yellow-green and shiny in life, the veins conspicuous and the lamina surface somewhat crinkled; trophophore stalk 1–30 percent of total trophophore length; blade outline narrowly oblong-triangular, up to 6 cm long and 2 cm wide; segments up to six pairs, strongly ascending, the

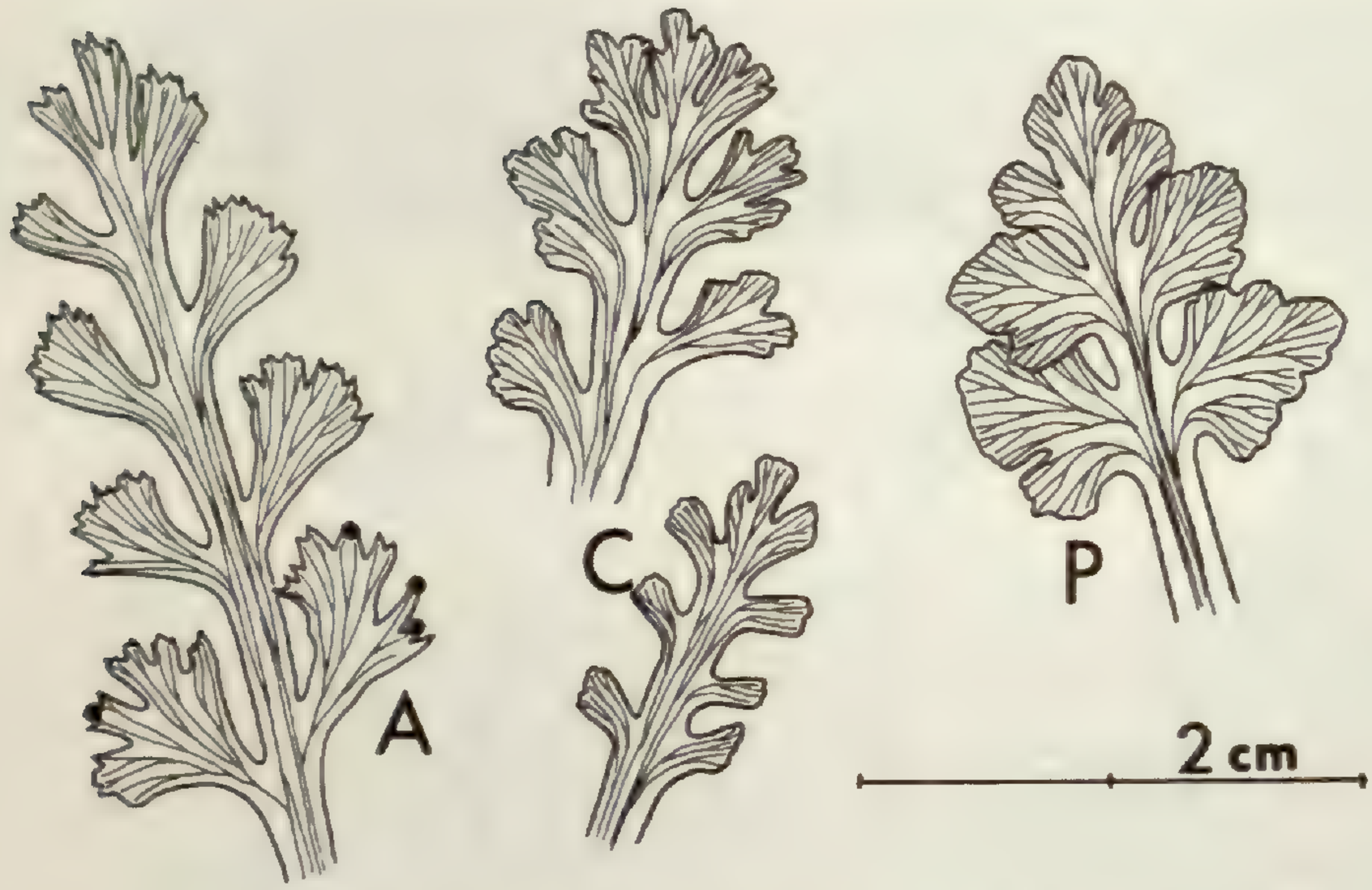


FIG. 2. *Botrychium* trophophore outlines and venation patterns. A. *B. ascendens, Wagner 81127*. C. *B. campestre, Wagner 85016*. P. *B. pedunculosum, Wagner 81124*.

upper ones extremely ascending; lower pinnae with somewhat curved posterior margins and straight anterior margins, commonly with marginal sporangia; largest pinnae 12 mm long, 10 mm wide; veins dichotomous, 2 or 3 major veins entering the pinna base, 14–17 vein endings along outer margins of larger pinnae; margins deeply and sharply dentate and serrate, often two- to five-lobed; sporophore branches mostly simple except for lower ones with 1–3 branchlets; spores finely verrucose, (40)44–47(54) μm in diameter; chromosomes $n = 90$.

Paratypes: CANADA. **Alberta:** Waterton Lakes National Park, Snowshoe Trail, ca. 1 mi NW of Red Rock Canyon Parking Area, Aug 1978, *R. Campbell* s.n. (MICH). **British Columbia:** Festuca Pass and lower SW slopes of Pipe Organ Peak, *Taylor et al. 5984, 5985* (UBC—mixed with *B. lunaria*). **Ontario:** Vicinity of mouth of Severn River, *Moir 1444* (CAN). **Yukon Territory:** Dawson, *Porsild 2040* (CAN—mixed with *B. lunaria*). U.S.A. **Montana:** Lewis and Clark Co., Sun River Primitive Area, E slope of China Wall near N end, 27 Jul 1948, *Hitchcock* s.n. (WTU). **Nevada:** Clark Co., Charleston Mts. [now Spring Mts.], Rainbow Falls, *Clokey & Clokey 7462* (UC—mixed with *B. lunaria*), *Clokey 8215* (all mixed with *B. lunaria*, DS, NY, UC, WTU), *Wagner 81150, 82115* (MICH); Charleston Peak, *Clokey 7463* (all mixed with *B. lunaria*, DS, NY, UC, WTU), *Clokey & Bean 7464* (UC—mixed with *B. lunaria*).

The epithet refers to the strongly ascending pinnae. This species is most similar to *B. crenulatum* Wagner (Wagner & Wagner, 1981) from which it differs in its narrowly cuneate segments (vs. broadly flabellate segments), sharply serrate margins (vs. shallow to strongly crenulate or dentate), a tendency for supernumerary sporangia on the lower pinnae (vs. no sporangia on the lower pinnae), more dilated and ascending terminal segments (vs. more skeletonized and spreading), relatively taller and more robust sporophore with more upright branches (vs. usually shorter sporophore with thinner and more spreading branches), and chromosome complement of $n = 90$ (vs. $n = 45$). A living plant is illustrated in Lellinger, 1985, figure 109. From *B. lunaria* and *B. minganense*, *B. ascendens* differs in a number of characters (cf. Wagner & Wagner, 1983a, figure 2: A = *ascendens*, C = *crenulatum*, L = *lunaria*, and M = *minganense*). It is interesting that there is no spore size increase with polyploidy; the tetraploid

FIG. 3. Presumed hybrid botrychiums with abortive spores. A. *B. pedunculosum* × *pinnatum*. OR: Wallowa Mts., Lostine River Valley, *Wagner 83361a*. B. *B. ascendens* × *crenulatum*, *Wagner 83363a*.

B. ascendens has spores only slightly different in size from the diploid *B. crenulatum* (see below, under *B. campestre*, the more striking contrast in spore size between that species and *B. minganense*).

Botrychium ascendens has a widely scattered range in western North America, but it is local and rare. In the type locality, in the Wallowa Mountains of Oregon, however, it occurs in hundreds along Hurricane Creek Trail in grassy fields interspersed with spruces. Growing with it are *B. crenulatum*, *B. lunaria*, and *B. minganense*.

Only a single hybrid (*Wagner 83363a*) was found. The other parent was deduced to be *B. crenulatum*. The pinnae of the hybrid average wider than those of *B. ascendens*, approaching the width of *B. crenulatum* (Fig. 3). The lowest pinnae are fan-shaped like those of *B. crenulatum*, but the remainder are cuneate as in *B. ascendens*. As expected, the spores of the hybrid turned out to be abortive. According to our present knowledge of the chromosomes of these plants, the hybrid should prove to be a sterile triploid.

Botrychium campestre W. H. Wagner & D. R. Farrar, sp. nov. (Figs. 2, 4, 5).—Type: U.S.A., Iowa, Plymouth Co., Five Ridge Prairie and Banks Tract, *Wagner 85016* (MICH).

Caulis subterraneus corporibus minutis fere sphaericis botryoideis munitus; segmenta parva linearia oblonga vel spatulata plerumque remota, segmentis maximis valde asymmetricis bifidisque; lamina oblongo-linearis, plerumque latissima ultra medium; sporophorum pro parte maxima trophophorum aequans vel parum superans: sporae parvae 34–38 μm diametro; chromosomatum numerus $n = 45$.

Plants exclusive of their roots 6(12) cm tall; common stalk usually 5(10) cm

FIG. 4. *Botrychium campestre*. Living plant in its native habitat, difficult to see among the grasses. Minnesota, Pipestone Co.

FIG. 5. *Botrychium campestre* leaves. Note short sporophores, narrow, remote, deeply incised pinnae, and broad midrib. Upper row, *Farrar s.n.*; lower row, *Eilers s.n.*

long; underground stems with tiny, nearly spherical bodies 0.4–0.8 mm in diameter, in grapelike clusters; sporophores stubby, usually equal to or slightly longer than trophophore but sometimes one-half or more longer; trophophore dull, whitish green, fleshy, the veins submersed; trophophore sessile (usually) to short-stalked, the stalk up to 10 percent of the total trophophore length; the stalk and midrib fleshy and 2–4 mm broad; blade outline oblong to linear oblong, commonly widest above the middle, up to 35 × 12 mm; segments linear to oblong to spatulate, the largest strongly asymmetrical and typically bifid, the lower lobe one-third to two-thirds the length of the upper lobe; number of pinna pairs few, usually less than six, these mostly remote; segments ascending mostly 30°–50°; largest pinnae reaching 7 mm long and 4 mm wide, served by two major veins

terminating on outer margin with 8–12 veinlets; the lowest pinnae generally narrower and less complex than the 1–3 distal pairs; outer margins mostly shallowly crenate or dentate; sporophore with all axes fleshy and flattened; spores finely verrucate, small, (30)34–38(44) μm, chromosomes $n = 45$.

Paratypes: CANADA. **Alberta:** Ft. Saskatchewan, near eastern edge, *Turner 7546* (ALTA); Ft. Saskatchewan, *Turner 8199* (ALTA—mixed with *B. minganense*). **Saskatchewan:** Beechy, *Ledingham & Hudson 992* (DAO); Dinsmore, *Ledingham & Hudson 1448* (USAS); 15 mi ENE of Fox Valley, *Ledingham & Jones 5676* (USAS). U.S.A. **Iowa:** Monona Co., Loess Hills Wildlife Area, 15 May, and 2 June 1982, *Eilers s.n.* (ISC, MICH); *Wagner 83207, 83208* (MICH). Plymouth Co., E of Banks Tract, *Farrar s.n.* (MICH). Woodbury Co., Sioux City, Briar Cliff College, Loess Hill Prairie, *Wagner 83205, 83206, 85014* (MICH); Stone State Park, *Wagner 85015* (MICH). **Minnesota:** Lac Qui Parle Co., Antelope Hills, *Wagner 85018* (MICH); Marietta, *Lyon 889* (MIN). Lincoln Co., Hole in the Mountain Prairie Preserve, *Wagner 85017* (MICH). Pipestone Co., Aetna Preserve, 2 mi NE of Holland, *Wagner 85019* (MICH). **Nebraska:** Brown Co., Niobrara Valley Preserve, *Freeman 1517* (KANU). **North Dakota:** McHenry Co., McHenry Sandy Prairie, *Stevens 1530* (US, UDA). McLean Co., 15 mi SSW of Velva, NE slope strip mine piles, 18 June 1974, *Disrud & Weber s.n.* (MICH).

The epithet refers to the occurrence of this species in grasslands and plains. Botanists, including ourselves, were much confused as to the identity of this moonwort, as shown by their annotations on herbarium sheets, viz., *B. boreale* var. *obtusifolium*, *B. dusenii*, *B. lunaria*, *B. lunaria* var. *minganense*, *B. matricariifolium*, *B. matricariifolium* var. *hesperium*, and *B. simplex*. *Botrychium campestre* is actually very distinctive once its characters are recognized. It resembles most *B. minganense*, with which it grows in at least one locality, and from which it can be separated by its much smaller, narrower, more dissected pinnae, fewer pinna pairs (in comparable-sized leaves), more fleshy and robust axes, diploid chromosome number, and spores 20–25 percent smaller than those of *B. minganense*. The peculiar round bodies attached to the stem are currently under investigation by D. R. Farrar and ourselves. They are evidently gemmae capable of propagating the plant; although root proliferations are known, specialized gemmae have never before been reported in Ophioglossales. Except for one questionable report, no vegetative reproduction has heretofore been known in *Botrychium*. Another feature, the early seasonal disappearance of the leaves, is different from all other known species of North American moonworts; in Iowa the leaves appear in April and turn brown and mostly die by mid-June.

Botrychium campestre was first discovered in Iowa by Theodore Van Bruggen and Lawrence Eilers in 1982. They called the plant to the attention of Donald R. Farrar, who, recognizing that it was unusual, referred it to us. Borrowed *Botrychium* specimens from the plains areas of the United States and Canada revealed that the prairie botrychium possesses a wide range, shown in Fig. 6. All localities so far are in prairies or prairie-like habitats, either in open grass or among shrubs. Data on labels read "moist sandy saline meadows," "dry west-facing loess prairie bluff," "sandy prairies," "gravelly shores of alkaline slough." The northwesternmost locality is a "north-facing, nearly bare, sandy side of south ditch of Canadian National Railroad, near the eastern edge of Fort Saskatchewan about 15 mi NE of Edmonton." Since many prairie plants occur along railroad tracks, collectors are urged to search for new localities in such places. The Prairie Moonwort is exceedingly rare and inconspicuous and takes much persistence and patience to find.

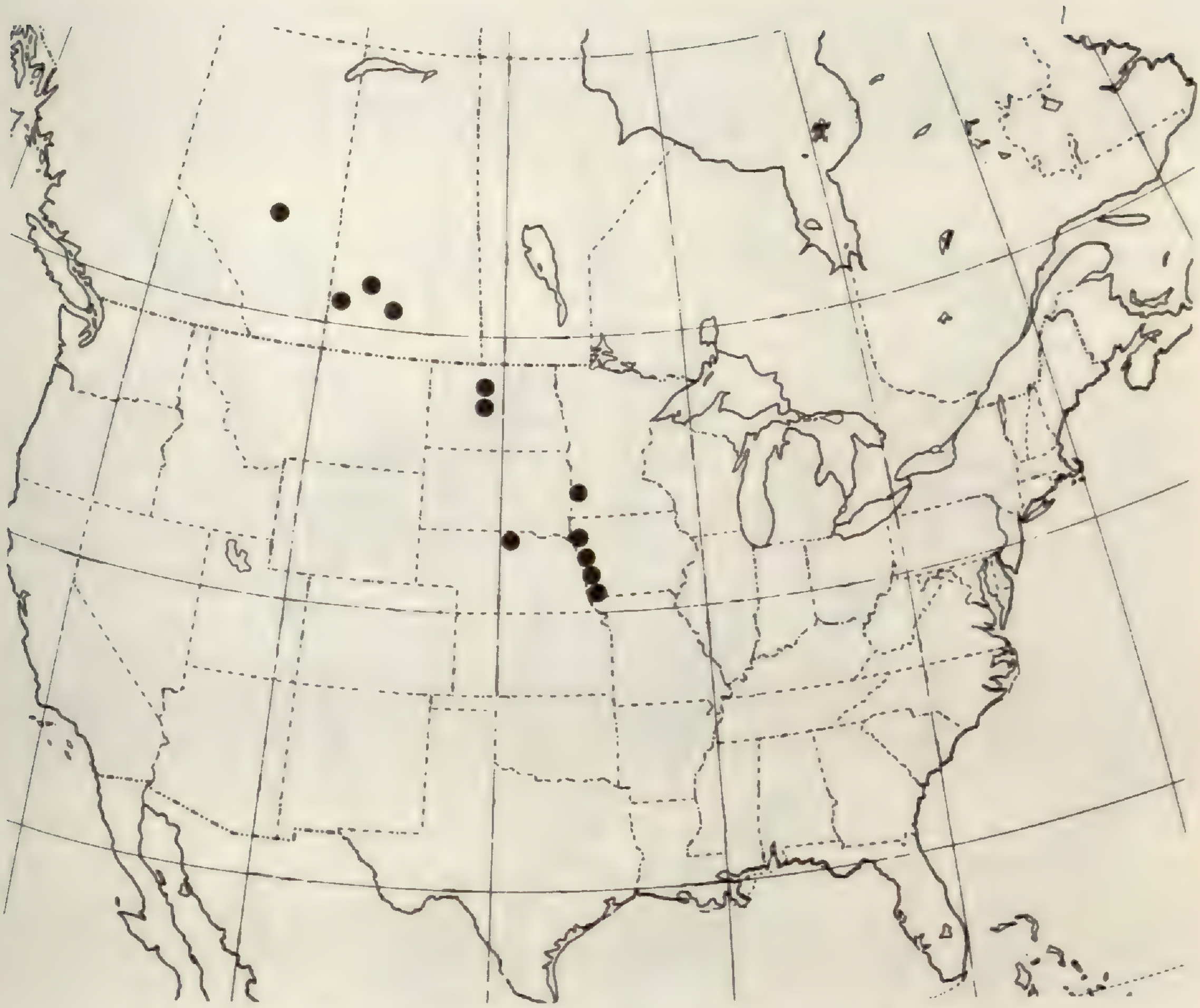


FIG. 6. *Botrychium campestre*. Range indicated by dots of known collections.

Botrychium pedunculosum W. H. Wagner, sp. nov. (Figs. 2, 7).—Type: U.S.A., Oregon, Wallowa Co., Lostine River Valley, 1.6 mi S of ranger cabin, 13.3 mi S of Lostine Church, grassy second-growth area along road, *Wagner 83361* (MICH).

Stipes communis maturus plerumque vitta angusta pallido-brunnea e trophophoro decurrenti instructa; stipes trophophori longus, 10–50% totae longitudinis trophophori; lamina trophophori triangularis vel ovato-triangularis; pinnae late affixae, plerumque approximatae, breves et angulares, grosse dentatae et plus minusve acutae; chromosomatum numerus $n = 90$.

Plants exclusive of roots (5)11–13(25) cm tall; common stalk usually with narrow pale brown stripe running down from the trophophore, (4)6–7(15) cm tall; sporophore (1.5)2(2.5) times as long as the trophophore; trophophore (in life) gray-green, dull, leathery, with veins submersed; trophophore stalk (10)30(50) percent of total trophophore length; blade (in life) commonly shallowly rolled, spoon-like, triangular to ovate-triangular, widest at the base, av. 4 cm long, 2.5 cm wide; pinnae somewhat ascending commonly approximate, (2)3(6) pairs, broadly attached, the lower ones asymmetrical, rhomboidal, angular, the tips usually more or less pointed, with 1–6 entire to coarsely crenate lobes, the upper ones becoming narrowly flabellate; largest pinnae up to 15 mm long and 10 mm wide;

FIG. 7. *Botrychium pedunculosum*, *Wagner 81124*. Note long trophophore stalks, and angular, deeply incised lower pinnae.

sporophore stalk (20)50(70) percent of sporophore length, sporophore branches in large plants with two major ascending lateral branches up to 70–100 percent as long as whole sporangial cluster; spores finely verrucate (36)37–41(43) μm diameter; chromosomes $n = 90$.

Paratypes: CANADA. **British Columbia:** E of Quesnel on road to Barkerville, *Calder et al. 14152*

(DAO—mixed with *B. pinnatum*); Rte. 26, near Quesnel, *Wagner 83339* (MICH); Rte. 26, 6.2 mi E of Victoria Creek, *Wagner 83347* (MICH). **Saskatchewan:** Cypress Hills, Hudson 1926 (DAO); Cypress Hills (Central Block), *Wagner 83309, 83111* (MICH). U.S.A. **Oregon:** Wallowa Co., Lostine River Valley, 1.2 mi N of Bowman Trail, *Wagner 81130* (MICH); type locality, *Wagner 81124* (MICH).

The epithet refers to the long stalk on the trophophore. The few collections of this species known outside of the Wallowa Mountains were identified as *Botrychium boreale* var. *obtusifolium* and *B. matricariifolium*. Mature plants of *B. pedunculosum* can be told readily from these taxa, as well as from *B. hesperium* and *B. echo* (cf. Wagner & Wagner, 1983a) by the usual presence on older plants of a well-defined, narrow, brown stripe running down the common stalk from the trophophore, by the unusually long trophophore stalk, and by the triangular-ovate blade with angular stubby asymmetrical segments. At the type locality, there are at least 200 large, healthy plants, growing together with *B. multifidum* (frequent), *B. lanceolatum* (abundant), *B. lunaria* (rare), *B. pinnatum* (frequent), *B. minganense* (frequent), and *B. simplex* (rare). A living plant is illustrated by Lellinger (1985, fig. 114). Where we have seen it in British Columbia, it occurs with the first four species in the foregoing list. Although it is widespread in western Canada, it is evidently very local and rare.

The hybrid, *Botrychium pedunculosum* × *pinnatum* (Fig. 3) was found among plants of *B. pedunculosum*, growing close to *B. pinnatum*. It merges the characters of the parents nicely: The plant is not so robust and broad as *B. pinnatum* nor so slender as *B. pedunculosum*. The trophophore of the hybrid shows the segmentation and dissection of *B. pinnatum* together with the more pointed pinnae and long stalk of *B. pedunculosum*. The sporophore is relatively short, as in *B. pinnatum*, but is divided into three major branches, as in *B. pedunculosum*. The single hybrid plant was readily recognized by its morphology and was found to have pronounced spore abortion as is usual in sterile interspecific crosses in this genus.

Discussion

With the description of the three taxa given above, we are able now to gain a better view of the endemism in the moonworts (subgenus *Botrychium*). It should be remembered that the common species of Europe and/or eastern North America, and their variations, are well known by hundreds of collections in the major herbaria of the world. They include *B. lunaria, B. lanceolatum, B. boreale, B. simplex, B. matricariifolium*, and *B. minganense*. Previously poorly known or unknown, the western North American taxa are well represented by ample collections for the first time. We thus have a substantial basis for taxonomic interpretation that we lacked before. And, more often than not, one or more of these taxa co-exist (Wagner & Wagner, 1983b) confirming their taxonomic distinctions. Accordingly, we can estimate the level of endemism.

Table 1 summarizes what we know about endemism in the moonworts. In spite of St. John's implication that the species tend to have world-wide distributions, there is only one, *B. lunaria*, about which that can be said. It is known not only throughout the boreal region but turns up again in Australia and New

TABLE 1. Endemism in *Botrychium* subg. *Botrychium* (based on Wagner & Wagner, 1981, 1983a; Wagner et al., 1984; and present paper).

Circumboreal and Amphitropical	Western North America
B. lunaria (L.) Swartz	*B. ascendens* Wagner
Circumboreal	*B. campestre* Wagner & Farrar
B. lanceolatum (Gmel.) Angstr.	*B. crenulatum* Wagner
B. simplex Hitchcock	*B. echo* Wagner
Northern and Alpine Eurasia	*B. hesperium* (Maxon & Clausen) Wagner & Lellinger
B. boreale Milde	*B. pedunculosum* Wagner
Amphiatlantic (Europe, e. N.A.)	*B. pinnatum* St. John
B. matricariifolium A. Br.	*B. montanum* Wagner
Northern North America	*B. paradoxum* Wagner
B. minganense Victorin	*B. ×watertonense* Wagner[1]
North Central North America	
B. mormo Wagner	

[1] Included because of its apparent ability to reproduce.

Zealand. Also the plant known as *B. dusenii* (Christ) Alston from southern South America may be the same as *B. lunaria* or a variety of it. As shown in the table, most of the species are limited to less than half of a continent. Some of them, like *B. campestre*, have much narrower ranges; and at least one, *B. mormo*, has a very small range. It is interesting to note that more than half of the world's species of moonworts are known only from western North America.

ACKNOWLEDGMENTS

This study was funded by National Science Foundation Grant BSR-82-2768, Monographic Studies of the genus *Botrychium*. The Iowa State Avisory Board of Reserves aided us in our field work in the vicinity of Sioux City in 1983. We are grateful to a number of persons who gave us assistance, especially Edward Alverson, W. R. Anderson, George Argus, David Bay, Dianne and William Blankenship, J. H. Hudson, and Donald R. Farrar. D. B. Lellinger and D. H. Wagner made helpful comments on the manuscript. The authorities of the Cypress Hills Provincial Park, Saskatchewan, and the Wallowa-Whitman National Forest, Oregon, kindly gave us guidance in the field. Special thanks go to Georgia Mason, who discovered that the Wallowa Mountains of northeastern Oregon harbor one of the richest North American *Botrychium* floras known. The following herbaria kindly supplied specimens: ALTA, DAO, DS, CAN, ISC, KANU, MIN, NY, UBC, UDA, US, USAS, WTU, and UC.

LITERATURE CITED

LELLINGER, D. B. 1985. *A field manual of the ferns and fern-allies of the United States and Canada.* Washington, D.C.: Smithsonian Inst. Press.

MASON, G. 1975. *Guide to the plants of the Wallowa Mountains of northeastern Oregon.* Eugene: Univ. Oregon Mus. Nat. Hist. Spec. Publ.

ST. JOHN, E. P. 1949. The evolution of the Ophioglossaceae of the eastern United States. Quart. J. Florida Acad. Sci. 12:207–219.

WAGNER, W. H., Jr., and F. S. WAGNER. 1981. New species of moonworts, *Botrychium* subg. *Botrychium* (Ophioglossaceae) from North America. Amer. Fern J. 71:20–30.

——— and ———. 1982. *Botrychium rugulosum* (Ophioglossaceae), a newly recognized species of evergreen grapefern in the Great Lakes area of North America. Contr. Univ. Michigan Herb. 15:315–324.

——— and ———. 1983a. Two moonworts of the Rocky Mountains; *Botrychium hesperium* and a new species formerly confused with it. Amer. Fern J. 73:53–62.

——— and ———. 1983b. Genus communities as a systematic tool in the study of New World *Botrychium* (Ophioglossaceae). Taxon 32:51–63.

———, ———, and J. M. Beitel. 1985. Evidence for interspecific hybridisation in pteridophytes with subterranean gametophytes. Proc. Royal Soc. Edinburgh 86B:273–285.

———, ———, C. Haufler, and J. K. Emerson. 1984. A new nothospecies of moonwort (Ophioglossaceae: *Botrychium*). Canad. J. Bot. 62:629–634.

CORRECTIONS TO *INDEX FILICUM*

Index filicum is now produced by the Royal Botanic Gardens, Kew, and as the new editor I am keen to correct the errors in the original and subsequent supplements. I am aware of a number of these but am sure that others are as yet undetected. I very much welcome information on any omissions and mistakes known to my colleagues. Infraspecific names are to be included in the next supplement, which will run from 1976–1985. From 1986, new names of pteridophytes will be published annually, as an appendix of an annual *Index Kewensis*, but *Index Filicum* will continue to be published at five- or ten-year intervals.—B. S. Parris, The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, England.

American Fern Journal 76(2):48–55 (1986)

Gametophytes of *Lycopodium lucidulum* from Axenic Culture

DEAN P. WHITTIER
Department of General Biology, Vanderbilt University, Nashville, TN 37235

TERRY R. WEBSTER
Department of Ecology and Evolutionary Biology, The University of Connecticut, Storrs, CT 06268

Lycopodium gametophytes are known from about 7% of the species (Bruce & Beitel, 1979). It is often difficult to find the subterranean gametophytes of *Lycopodium* in nature. One way of increasing the number and kinds of subterranean gametophytes for study is to grow them from spores in axenic culture. Two types of subterranean *Lycopodium* gametophytes, carrot- and disc-shaped, have been grown in axenic culture on nutrient media containing minerals and sugar. The structure of these gametophytes was essentially the same as that for gametophytes from nature (Whittier, 1977, 1981).

Lycopodium lucidulum represents a third type of subterranean gametophyte. Its gametophytes are axial, white, and fleshy (Spessard, 1922) and do not resemble the disc- or carrot-shaped gametophytes of *L. obscurum* and *L. digitatum* respectively which have been previously grown in culture (Whittier, 1977, 1981). The main goal of this study was to grow this third type of subterranean *Lycopodium* gametophyte in axenic culture, and to compare its morphology to gametophytes of the same species from nature.

MATERIALS AND METHODS

Plants of *Lycopodium lucidulum* Michx. bearing sporangia were collected from various places in the northeastern United States and Tennessee in early fall. Vouchers are on deposit in the Vanderbilt University Herbarium (VDB). Spores were obtained by allowing fertile branches to dry over sheets of paper. The spores were surface sterilized with 20% Clorox by the method of Whittier (1964) and were sown on 15 ml of nutrient medium in culture tubes with screw caps that were then tightened. The nutrient medium was composed of Knudson's solution of mineral salts, FeEDTA, 0.5% sucrose, and 0.6% agar. The spores were cultured at 24 ± 1°C in darkness or in light, 1250 lux, from Gro-lux fluorescent lamps for 12 of every 24 hours.

The gametophytes studied were first photographed and then fixed. Fixation from culture was accomplished with a 1:1 mixture of 4% glutaraldehyde and 10% acrolein which were both in 0.07 M phosphate buffer at pH 6.8. Gametophytes from nature were fixed in FAA. After fixation, the gametophytes and pieces of gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin, and fast green. Hand sections of unfixed gametophytes

were stained with alcian blue (1% in 3% glacial acetic acid) to demonstrate mucilage (Pearse, 1968).

Results

Efforts to observe the early stages of spore germination after the spores had been in culture for 6–12 months were unsuccessful. Cultures that had been in the dark for 2 or more years were the source of gametophytes for this investigation. In these older cultures, one to a few gametophytes were usually found in most tubes. No gametophytes were found in cultures maintained in the light.

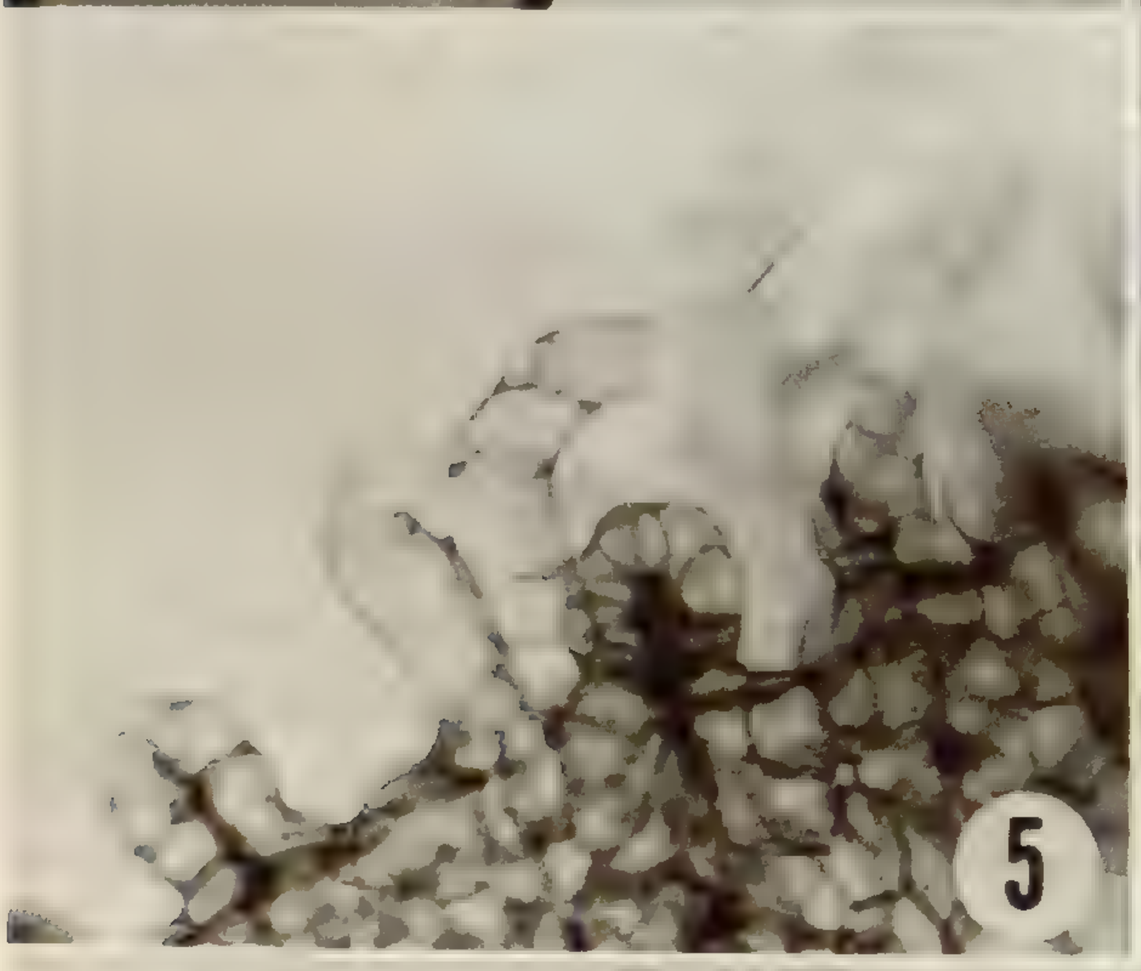
The gametophytes of *L. lucidulum* from axenic culture are axial structures (Figs. 1, 3). However, they are slightly flattened with distinctive dorsal and ventral surfaces (Figs. 2, 4, 9). The dorsal and ventral surfaces are separated by indentations along the lateral surfaces of the gametophyte (Figs. 3, 9). The dorsal surface is covered with short paraphyses which obscure the sex organs (Figs. 2, 3, 4, 5, 9). Although the paraphyses may be completely uniseriate, often the basal portions are biseriate where they attach to the gametophyte (Fig. 5). If the paraphyses have a biseriate base, that portion branches to form two uniseriate hairs (Fig. 5). The ventral surface has rhizoids which are coated with a mucilaginous material. The mucilage stains with alcian blue (Fig. 6), indicating that the rhizoid mucilage is composed of an acid mucopolysaccharide.

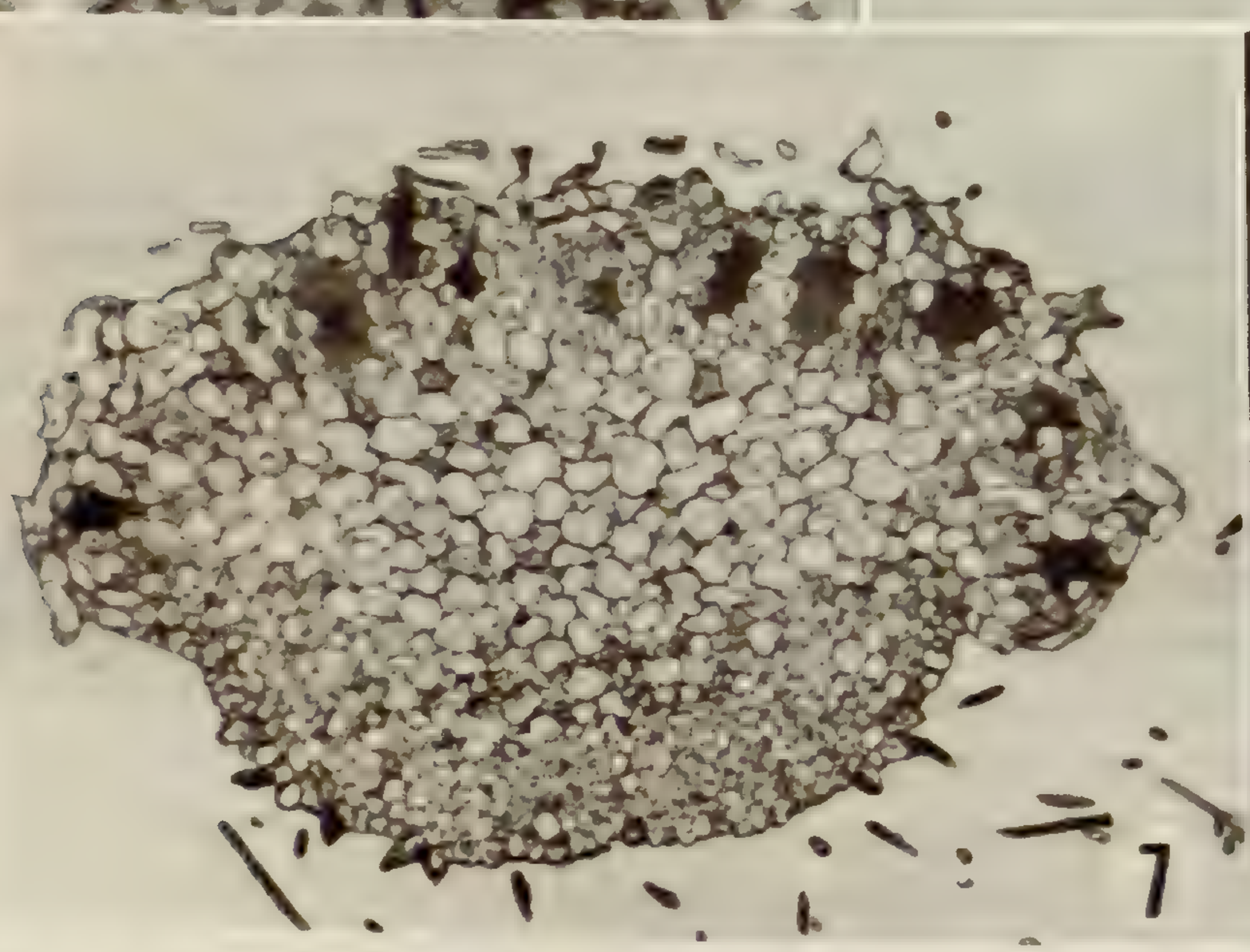
The youngest (smallest) gametophytes from culture have the dorsiventral growth habit (Figs. 8, 9). Even with these small gametophytes, the indentations on the lateral surfaces extend for almost their whole length. Thus, it appears that the mature growth habit is established at a small size and early age in the gametophytes of *L. lucidulum*.

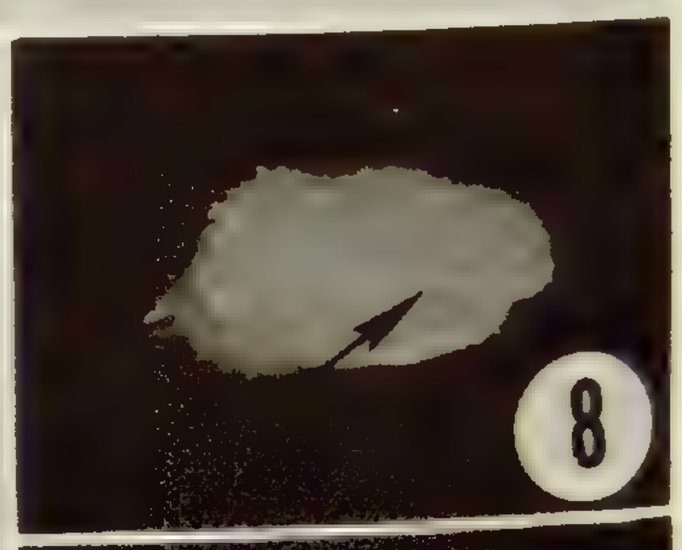
The meristem is located in a groove on the lower surface of the apical region (Figs. 4, 8, 9, 10). The derivatives to the upper side of the meristem form the dorsal tissues including sex organs and paraphyses (Figs. 4, 10). The immature dorsal tissues overarch the meristematic region and cause the meristematic groove to be on the lower surface of the apical region (Figs. 4, 10). The meristematic derivatives to the lower side form the ventral portion of the gametophyte including unicellular rhizoids. The meristematic groove is continuous with the indentations on the lateral surfaces of the gametophyte (Fig. 9).

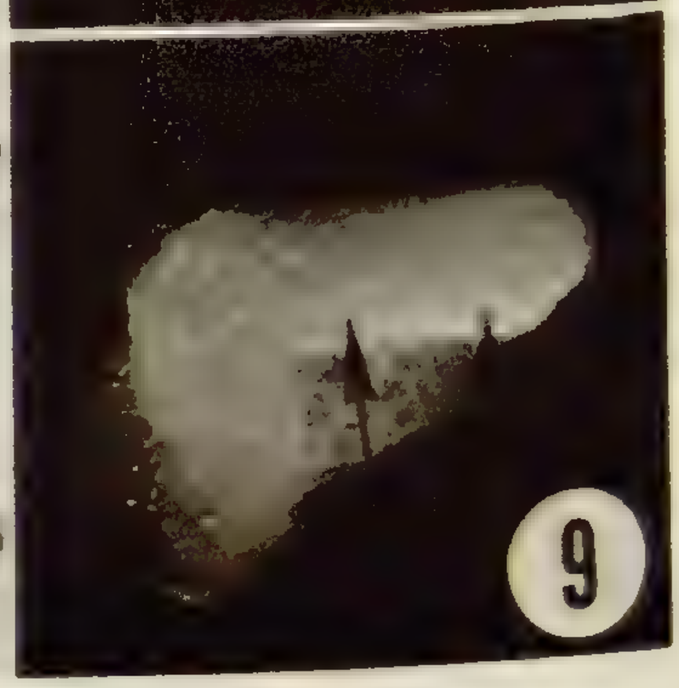
The apical region of the gametophyte is usually about the same width as the mature portion of the gametophyte, especially the mature region immediately basipetal to the apex. Thus, it appears that the meristem has limited activity on its lateral margins in the apical region. Gametophytes (Fig. 4) with expanding apical regions appear to be ones which are preparing to branch. A few branched gametophytes were found in the cultures.

The gametangia are initiated in the apical region from recent derivatives of the meristem (Fig. 10). In mature gametophytes, a gradation of gametangial developmental stages from initiation to maturity exists in the dorsal tissue of the apical region. Stages in the development of antheridia are illustrated in Figure 10. The antheridia are mature once their position has shifted to the top of the dorsal lip. The mature antheridia are large and sunken and have only a few









FIGS. 1–9. Gametophytes of *L. lucidulum* from axenic culture. FIG. 1. Gametophyte on surface of nutrient medium, ×1.5. FIG. 2. Dorsal surface of gametophyte with rough surface caused by paraphyses, ×4.5. FIG. 3. Lateral view of gametophyte. Ventral surface to left of lateral indentation (arrow) and dorsal surface to right, ×7. FIG. 4. Ventral view of apical region. Meristematic groove (arrows) below overarching dorsal tissue, ×6. FIG. 5. Section of dorsal surface with archegonia and paraphyses, ×120. FIG. 6. Rhizoid tips with mucilaginous sheaths stained with alcian blue, ×600. FIG. 7.

jacket cells at the gametophyte surface (Fig. 10). It appears that during fixation sperm are occasionally released and masses of sperm can accumulate on the surface of cultured gametophytes (Fig. 10).

The position and development of the archegonia have the same relationship to the apex as the antheridia. The archegonial necks are not obvious on the whole gametophytes because of the dorsal paraphyses. The necks are of a medium length for *Lycopodium* (Figs. 5, 11). Most of the archegonia have 4 neck canal cells and 4 tiers of exposed neck cells (Fig. 11).

Rarely are the antheridia and archegonia interspersed along the gametophytes from culture. Young gametophytes have only antheridia, and older gametophytes eventually form archegonia. Transition regions do occur between areas with all archegonia or all antheridia in which both gametangia are present. However, even in these regions there is little intermixing of the gametangia. Usually in these transition regions one type of gametangium has a median distribution and the other type has a more lateral position. It appears that the change in type of gametangium formation occurs in the middle of the apical meristem first and spreads laterally.

The internal tissues of the gametophytes from culture do not have the mycorrhizal fungus (Figs. 7, 10) which is found in the ventral tissues of gametophytes from nature (Fig. 12). The internal tissues are composed of numerous layers of isodiametric cells (Figs. 7, 13) and no palisade or vertically elongated cells occur.

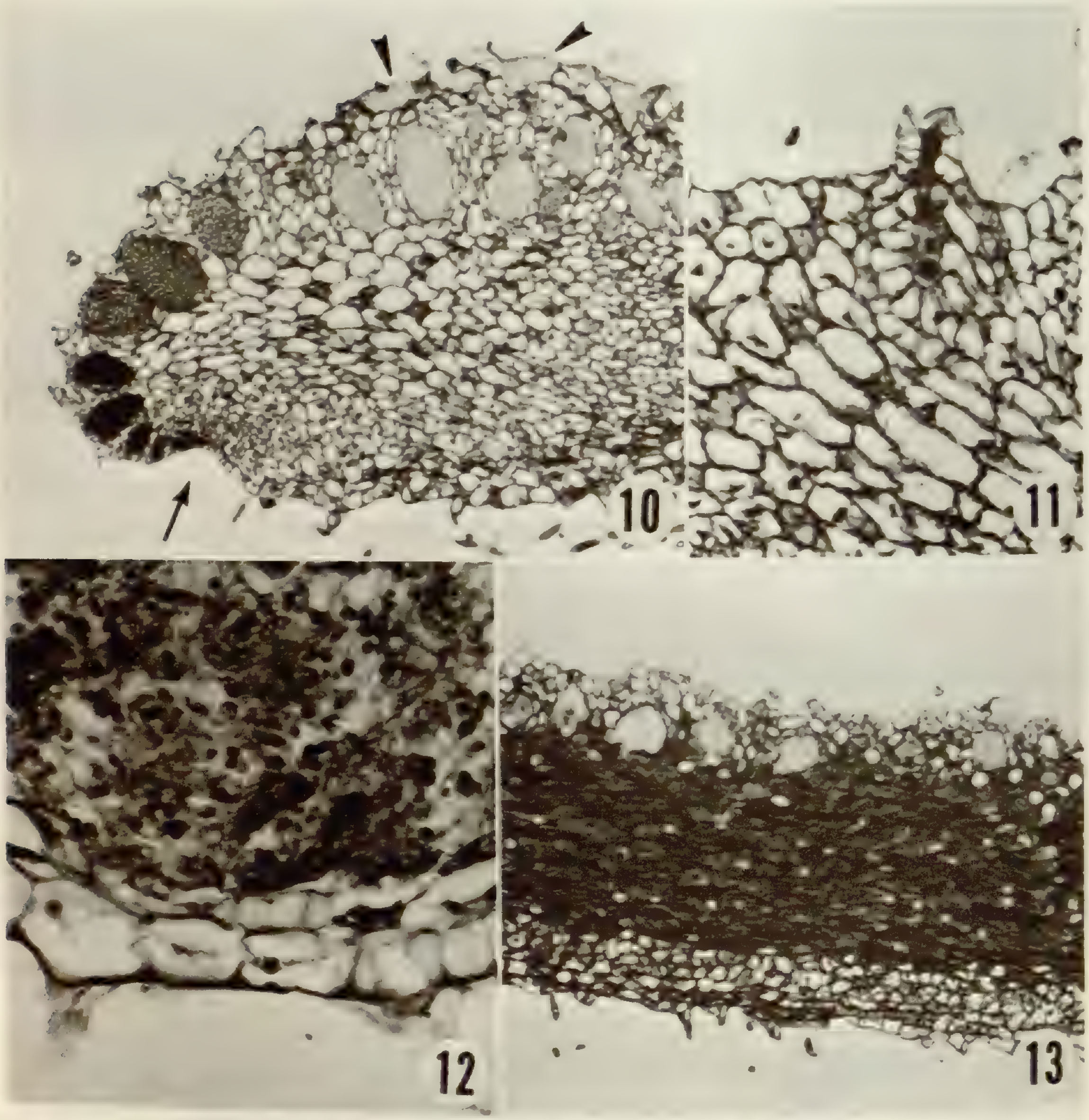
Although the internal tissues of most of the cultured gametophytes are rather uniform (Fig. 7), some gametophytes contain cells with distinctive contents (Fig. 13). In these cases the central cells contain large amounts of a fine grain, blue-staining, condensed tannin (Fig. 13). In addition, these cells have large amounts of starch, although it should be noted that the other internal cells do contain some starch. The surface cells in the gametangial region and 5 or 6 layers of the most ventral cells lack the condensed tannins (Fig. 13).

Discussion and Conclusions

The percentage of spore germination was very low, less than 0.1%, and the early stages of germination were not observed in these cultures. There may have been so few germinating spores that they were missed when the cultures were sampled at 6 and 12 months. However, it is possible that the spores of *L. lucidulum* take longer than a year to germinate. Bruchmann (1910) has reported that spores of some species of *Lycopodium* take 3 or more years to germinate. A delayed germination would help to explain why the gametophytes of *L. lucidulum* were only found in 2–3 year old cultures.

←

Cross section of gametophyte with antheridia and paraphyses above the lateral indentation and the rhizoid-bearing surface below, ×50. Fig. 8. Lateral view of young gametophyte with overarching dorsal tissue in the apical region and the lateral indentation (arrow), ×8. Fig. 9. Lateral view of young gametophyte with lateral indentation (arrows) between the rhizoid-bearing ventral surface and the paraphyses-bearing dorsal surface, ×5.



FIGS. 10–13. Structural details of gametophytes of *L. lucidulum*. FIG. 10. Sagittal section through apical region of gametophyte with meristem (arrow) and numerous developmental stages of antheridia in the dorsal tissues. Sperm on dorsal surface (arrowheads), ×65. FIG. 11. Section of dorsal surface with archegonium having egg and four neck canal cells, ×125. FIG. 12. Section of gametophyte from nature with mycorrhizal fungi in ventral tissue, ×600. FIG. 13. Sagittal section of gametophyte with an accumulation of condensed tannins in some cells, ×45.

The germination of spores of *Lycopodium* species with subterranean gametophytes has been poor in axenic culture. For the three species, *L. lucidulum*, *L. digitatum*, and *L. obscurum*, that have been germinated in culture (Whittier, 1977, 1981), germination has not exceeded 0.1%. Experimental studies are underway on spore germination in other species of *Lycopodium*. Establishing techniques for accelerating germination and/or increasing the percentage of germination would greatly enhance studies on the subterranean gametophytes of *Lycopodium* in axenic culture.

The gametophytes of *L. lucidulum* from axenic culture were compared with the descriptions of gametophytes from nature by Spessard (1922) and Bruce and

Beitel (1979). In addition these cultured gametophytes were compared with field-collected gametophytes from a wooded area in Storrs, Connecticut, one of the sites from which spores were obtained. These comparisons show that the basic organization of the cultured gametophytes is the same as that for the gametophytes from nature.

A major difference between gametophytes from the two sources is the absence of a mycorrhizal fungus from the cultured gametophytes. These gametophytes develop on a nutrient medium containing minerals and sucrose. Thus, the culture medium supplies the necessary nutrients, especially the organic material, which the gametophytes from nature obtain from the mycorrhizal fungus. Like other subterranean, pteridophyte gametophytes grown in axenic culture (Whittier, 1983; Whittier & Peterson, 1984), gametophytes of *L. lucidulum* develop without the mycorrhizal fungus if a carbon source is supplied.

The gametophytes from nature and culture have a thickened, axial morphology with the possibility of apical branching. The apices of the gametophytes from both sources are the same, with the meristematic groove being overarched by a lip of immature dorsal tissue. In culture the dorsal and ventral surfaces are separated by an indentation along the lateral surfaces of the gametophyte. Although there appears to be no growth associated with the lateral indentations on the gametophytes from culture, they are continuous with the meristematic groove in the apical region. The gametophytes from nature, especially the larger ones, are partially to almost completely boat-shaped. It would appear that growth along the sides of these gametophytes, if only in the apical region, causes the ventral surface to be displaced upward along the lateral margin. The thickness of this marginally uplifted ventral tissue produces the boat-shape. This shape has not been observed in the cultured gametophytes, possibly because they are younger and smaller than those from nature.

The morphology of the sex organs and their placement is the same on gametophytes from both sources. The dorsal surface of the cultured gametophytes also have the paraphyses reported for gametophytes from nature (Spessard, 1922; Bruce & Beitel, 1979). The paraphyses are apically uniseriate but usually arise in pairs from biseriate bases. Spessard (1922) suggested that these paraphyses are mucilaginous. The paraphyses on the cultured gametophytes give no indication of mucilage production. There is no mucilage formed by cells on the dorsal surface of the cultured gametophytes, although the rhizoids on the ventral surface have mucilaginous sheaths. The only viscid material found on the dorsal surface of some of the cultured gametophytes was sperm released during fixation.

The internal structure of the cultured gametophytes is typical for the species. The cells in the ventral region are isodiametric, which is similar to the condition for gametophytes from nature. The radially elongated cells of the inner mycorrhizal zone of the gametophytes of *L. digitatum* (Bruce, 1979) and *L. obscurum* (Spessard, 1922) are not present in the gametophytes of *L. lucidulum*. The mycorrhizal zone in *L. lucidulum* is similar to that found in *L. selago* (Bruchmann, 1910), in which the fungus occurs in the 5 or 6 layers of cells just internal to the ventral surface cells.

In most of the sections of cultured gametophytes, the internal cells were sim-

ilar to each other. However, in some gametophytes there were differences in the contents of the internal cells. Five or six layers of cells in the ventral region lacked the large amounts of condensed tannins present in the more central and dorsal tissue. Except for differences in the amounts of the condensed tannins, the tissues of these gametophytes appear normal. Why all the gametophytes did not exhibit this condition is unknown. However, these sections appear to demonstrate that there is a potential difference in the ability of the internal cells to synthesize polyphenolic material or condensed tannins. The distribution of the condensed tannins in these gametophytes is of interest because the cells lacking condensed tannins are those which in gametophytes from nature contain the mycorrhizal fungus. Additional studies are needed to determine under what conditions the differences in the polyphenolic contents of the cells occur and to determine if the high amounts of polyphenolic materials usually correlate with the non-mycorrhizal portions of the gametophyte.

The studies on *Lycopodium* gametophytes from axenic culture, which now include three types of the subterranean gametophytes, demonstrate that a mycorrhizal fungus is not necessary for gametophyte growth. As long as the nutrient medium contains sugar, the gametophytes develop in culture without a mycorrhizal fungus. The morphology and anatomy of these gametophytes from culture are essentially the same as for those from nature. Even though no fungus is present, the mycorrhizal zone differentiates in *L. obscurum* and *L. digitatum* and, in those gametophytes of *L. lucidulum* with large amounts of condensed tannins, the mycorrhizal zone is distinctive. These observations indicate that the internal and external structure of *Lycopodium* gametophytes is an inherent characteristic of the gametophytes and not dependent on the mycorrhizal fungus.

ACKNOWLEDGMENTS

The authors thank Dr. William G. Eickmeier and Dr. Dominick J. Paollilo for spore collections and Dr. R. James Hickey for gametophytes from nature. This study was supported in part by the Vanderbilt University Research Council.

LITERATURE CITED

BRUCE, J. G. 1979. Gametophyte of *Lycopodium digitatum*. Amer. J. Bot. 66:1138–1150.

——— and J. M. BEITEL. 1979. A community of *Lycopodium* gametophytes in Michigan. Amer. Fern J. 69:33–41.

BRUCHMANN, H. 1910. Die Keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L., *L. annotinum* L., und *L. selago* L. Flora 101:220–267.

JOHANSEN, D. A. 1940. *Plant microtechnique*. New York: McGraw-Hill Publications.

PEARSE, A. G. E. 1968. *Histochemistry theoretical and applied*. 3rd ed. Vol. 1. Baltimore: Williams and Wilkins Co.

SPESSARD, E. A. 1922. Prothallia of *Lycopodium* in America II. *L. lucidulum* and *L. obscurum* var. *dendroideum*. Bot. Gaz. (Crawfordsville) 74:392–413.

WHITTIER, D. P. 1964. The effect of sucrose on apogamy in *Cyrtomium falcatum* Presl. Amer. Fern J. 54:20–25.

———. 1977. Gametophytes of *Lycopodium obscurum* as grown in axenic culture. Canad. J. Bot. 55:563–567.

———. 1981. Gametophytes of *Lycopodium digitatum* (formerly *L. complanatum* var. *flabelliforme*) as grown in axenic culture. Bot. Gaz. (Crawfordsville) 142:519–524.
———. 1983. Gametophtyes of *Ophioglossum engelmannii*. Canad. J. Bot. 61:2369–2373.
——— and R. L. PETERSON. 1984. Gametophytes of *Botrychium lunarioides* and their mucilage-coated rhizoids. Canad. J. Bot. 62:2854–2860.

SHORTER NOTE

A New Substrate for *Ophioglossum palmatum* in Florida.—*Ophioglossum palmatum* L. occurs on a variety of substrates throughout most of its geographic range (Mesler, Amer. Fern J. 64:33–39, 1974, and references therein). In Florida, the species has been reported by all previous authors to occur exclusively as an epiphyte among leaf bases of the Cabbage Palm [*Sabal palmetto* (Walt.) Lodd. ex Schultes]. We are here reporting its occurrence on another species, Saw Palmetto [*Serenoa repens* (Bartr.) Small]. The plants were first found by Steve Farnsworth in Martin County (T39S, R40E, secs. 28–33). The site is a mixture of Pine flatwoods and Cabbage Palm hammock with scattered portions of Oak hammock. A unique combination of factors has allowed *O. palmatum* to colonize the Saw Palmetto including: 1) A large nearby population of *O. palmatum* growing on Cabbage Palms; this indicates proper climatic and site-specific conditions and serves as an unusually sizable spore source for the species. 2) The presence of an unusual arborescent form of the Saw Palmetto, with trunks to nearly 3 m tall and an ample number of leaf bases available for colonization. 3) The absence of fire for probably 20–30 years. The site is bordered by a road to the south, a canal to the north, and by orange groves and houses to the west and east—all impediments to fire. This absence of fire is probably the most important factor. It contributes, at least in part, to the existance of both the spore source populations and the arborescent form of the Saw Palmetto.

The site is on private property and currently being cleared for cattle grazing. We understand that burning is not planned; therefore the plants may persist at the site for some time. The ferns were present on at least six separate Saw Palmettos and more than 20 Cabbage Palms within a single one acre tract. One of us (R.M.) has transplanted several Saw Palmettos and Cabbage Palms (that had otherwise been marked for cutting) bearing *O. palmatum* to his nursery.

The occurrence of *O. palmatum* on a new substrate does not appear to be a sign of recovery by this rare and endangered species. Saw Palmettos ordinarily occur in habitats with a natural fire cycle. Since fire effectively eliminates both the plants of *O. palmatum* and their microhabitat, we do not expect this phenomenon to be frequent in Florida. A decrease in natural fires in areas near human habitation or agricultural lands may slow the decline in numbers that *O. palmatum* has been experiencing, though it is doubtful that this will offset losses due to direct habitat destruction by humans.—CLIFTON E. NAUMAN, Fairchild Tropical Garden, 10901 Old Cutler Rd., Miami, FL 33156, and RICHARD MOYROUD, 202 Grove Way, Delray Beach, FL 33444.

American Fern Journal 76(2):56–98 (1986)

Revision of the Neotropical Fern Genus *Cyclodium*

ALAN R. SMITH
Department of Botany, University of California, Berkeley, CA 94720

In recent years, the tropical American genus *Cyclodium* Presl (Dryopteridaceae) has been regarded as comprising two species (Christensen, 1905–1906; Copeland, 1947), a subgroup of *Dryopteris* comprising five species (Morton, 1939), or congeneric with *Stigmatopteris* (Kramer, 1978; Tryon & Tryon, 1982), with about 20 species. It has been variously allied to *Phanerophlebia* (Copeland, 1947), *Ctenitis* and *Tectaria* (Tryon & Tryon, 1982, p. 520), *Soromanes* (a segregate of *Polybotrya*; Christensen, 1938; Pichi Sermolli, 1977), and *Thelypteris* subgenera *Goniopteris* and *Meniscium* (Morton, 1939).

A study of the constituent species, as well as allied genera, convinces me that none of these circumscriptions is tenable. In this paper, I admit six additional known species within its circumscription and describe two new ones. Thus defined, *Cyclodium* comprises 10 species and has its closest alliance with neither *Dryopteris* nor *Stigmatopteris* but probably with *Polybotrya*, also an exclusively American genus. The relationship of *Cyclodium meniscioides*, the type of the genus, with certain other species, particularly *C. calophyllum*, *C. heterodon*, and *C. varians*, is so close that one has some difficulty separating them specifically. There is also some evidence that hybridization is taking or has taken place to produce both sterile hybrids and possibly fertile allopolyploid derivatives. The more dissected and free-veined species (*C. trianae*, *C. seemannii*) are connected to those with anastomosing veins through *C. inerme* and agree with the type in microscopic details of indument, blade architecture, indusium, spores, and venation.

TAXONOMIC HISTORY

Cyclodium was described in 1833 by Presl, who admitted three species: *C. glandulosum* (Blume) Presl [type from Java; ≡*Pronephrium glandulosum* (Blume) Holttum, a member of the Thelypteridaceae]; *C. meniscioides* (Willd.) Presl; and *C. confertum* (Kaulf.) Presl [=*C. meniscioides*]. He later recognized a fourth species, *C. abbreviatum* Presl [based on *Aspidium abbreviatum* Schrader]. Moore (1857–1862) transferred two additional species to *Cyclodium*: *C. heterodon* (Schrader) Moore and *C. cumingianum* (Presl) Moore [≡*Anisocampium cumingianum* (Presl) Moore, an athyrioid genus]. Hooker (1862) and Hooker and Baker (1867) treated *Cyclodium* as a section of *Aspidium* and included also *Aspidium hookeri* Baker [=*Amphineuron opulentum* (Kaulf.) Holttum, a thelypteroid].

Christensen (1913, 1920) included three species (*varians*, *guianensis*, *sanctigabrieli*) as members of his *Dryopteris* subg. *Stigmatopteris* group *Peltochlaena*, but he never realized the connection between these species and *C. meniscioides*. *Dryopteris trianae* was placed by Christensen in subg. *Polystichopsis*, together

with species now regarded as belonging to *Arachniodes*, *Polystichopsis*, and *Lastreopsis*.

Morton (1939) realized the relatively close relationship between *C. meniscioides*, with imparipinnate laminae, and species with a confluent, pinnatifid apex (*varians*, *calophyllum*), but he also included a species of *Thelypteris* subg. *Goniopteris* (*Dryopteris clypeata* Maxon & Morton) in this alliance.

Kramer (1978) and Tryon and Tryon (1982) combined *Cyclodium* and *Stigmatopteris* in a single genus and used the later name *Stigmatopteris* in anticipation of its conservation. This proposal (by Tryon & Tryon, 1981) was rejected by the Committee for Pteridophyta (Pichi Sermolli, 1982).

No one appears to have questioned the artificiality of *Stigmatopteris* as defined by Christensen (1909, 1913), even though Christensen recognized two distinct groups (*Eustigmatopteris* and *Peltochlaena*) based on lamina texture and the presence or absence of indusia. It is my belief that these two groups have no close relationship and should not be considered congeneric. *Stigmatopteris* is construed here in a narrow sense to comprise those species 12–25 of Christensen's (1913) revision, along with a few others more recently described.

Materials and Methods

This revision is based almost entirely on the study of approximately 1200 collections (including duplicates) representing about 475 gatherings from 26 herbaria. I am grateful to curators and staff of the following institutions for loans of specimens: AAU, B, BM, BR, C, CAY, COL, F, FI, G, GB, GH, K, M, MICH, MO, NY, P, PORT, RB, U, US, VEN, W, Z.

Peter Edwards has kindly sent fixed chromosome material from specimens of *Cyclodium* grown at Kew. He has also provided important information on the habit and distribution of one of the new species, *C. akawaiorum*, which was collected on a recent expedition to Mt. Roraima.

Spore samples for SEM were mounted on aluminum stubs using television tube coat. Mounted samples were allowed to dry for 24 hours before being coated with approximately 25 nm gold using a Polaron V sputter coater. SEM was done on an ISI DS-130 at an accelerating voltage of 8 or 10 kV. Facilities were provided by the Electron Microscope Laboratory, Life Sciences Building, University of California, Berkeley. Nelson Barton performed the sample preparation and microscopy.

I thank Colleen Sudekum for executing most of the drawings of individual species. Charlotte Hannon provided the drawings of *Cyclodium akawaiorum* and aided in preparation of the distribution maps. Correspondence with Robbin Moran, Illinois State Natural History Survey, on *Cyclodium* and related genera resulted in discussion and insight into intergeneric relationships.

Morphology

Rhizome and scales.—Rhizomes of *Cyclodium* species are woody, dictyostelic, and long- to short-creeping. Internode length is correlated with habit, with those

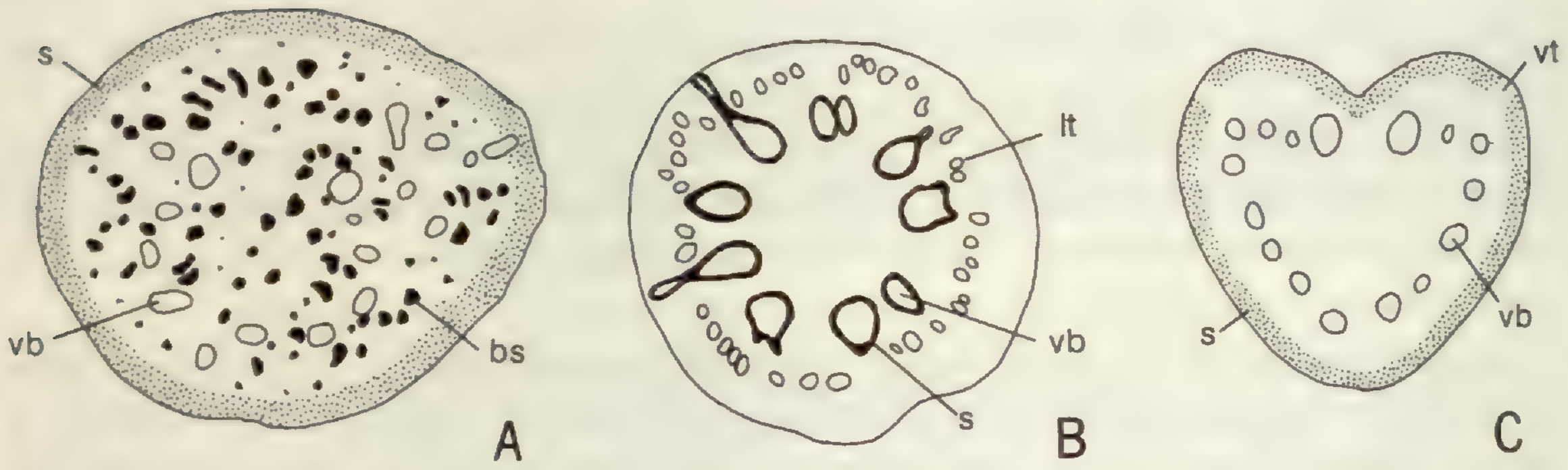


FIG. 1. Rhizome and stipe sections in *Cyclodium* and *Polybotrya*. A. Rhizome of *Cyclodium inerme*, *Steyermark et al. 122169*, UC. B. Rhizome of *Polybotrya serratifolia* (Fée) Klotzsch, *Smith 922*, UC. C. Stipe base of *Cyclodium guianense*, *Liesner & González 10375*, UC. s = sclerenchyma; vb = vascular bundle; vt = ventilation band; bs = brachysclereid; lt = leaf trace.

species that are hemiepiphytic (*C. meniscioides*, *C. akawaiorum*, *C. varians*) having longer creeping rhizomes and more distant stipes than those that are terrestrial. Individual meristeles are not surrounded by a blackened sclerenchymatous sheath, are more variable in size, and are arranged in a more irregular ring (Fig. 1A) than in species of *Polybotrya* (Fig. 1B). Numerous patches of blackened sclerenchymatous cells (brachysclereids) dot the stele throughout, at least in older rhizomes of *Cyclodium* (Fig. 1A).

The rhizomes bear densely overlapping, linear-lanceolate scales at the apex, but these become less visible and abraded back from the apex. In general, the scales are brown with a denticulate margin; sometimes they have a slightly darkened midband. They are subentire to denticulate along the margin (Figs. 6E, 7F, 9H). These scales are somewhat similar to those in some species of *Polybotrya* and are readily distinguished from scales of most *Dryopteris* and *Stigmatopteris* species, which have scales that tend to be broader, less rigid, and more uniform in color.

Axis vasculature and sulcation.—In cross section, stipes of *Cyclodium* have numerous (generally 8–16) round to oval meristeles arranged in a ring, with the two largest bundles being at the adaxial side of the ring (Fig. 1C). This pattern is typical for dryopteroid ferns and contrasts markedly with the pattern observed in athyrioid and thelypteroid ferns, where there are generally only two vascular bundles in the stipe. The outer portion of the stipe consists of a band of sclerenchyma that is interrupted only by two relatively narrow ventilation bands along the adaxial flanks.

Fronds of *Cyclodium* are anadromous (i.e., the lamina is polystichoid; Christensen, 1920), with the acroscopic segment arising before the basiscopic one (Fig. 13C, D, F, G). This is true of both proximal and medial pinnae. In the case of simply pinnate species, the first main lateral vein arises acroscopically (Figs. 7K; 11B, C, F). Anadromous architecture is the primitive condition in the closely related genera *Polybotrya* (Moran, in litt.) and *Maxonia*, and also in the less closely related genera *Polystichopsis* and *Arachniodes*.

Axes of *Cyclodium* are slightly to deeply grooved adaxially, and the grooves of the costae are more or less continuous with grooves of the main rachis. This

pattern is like that of most dryopteroid genera and differs from that found in tectarioid genera such as *Ctenitis* and *Tectaria*, which have axes raised or flattened adaxially.

Lamina.—The texture of the lamina in *Cyclodium* is usually chartaceous to subcoriaceous and rather firm. In this feature it is similar to *Polybotrya*, *Olfersia*, and *Maxonia*, but it differs from *Stigmatopteris*, which generally has thinner laminae. *Cyclodium* also lacks the internal glands (translucent areas in the lamina) characteristic of most *Stigmatopteris* species. Laminae vary from 1-pinnate to 2-pinnate or more divided. More dissected species (*C. seemannii*, *C. trianae*) greatly resemble certain species of *Polybotrya* (e.g., *P. caudata*). Even the less divided ones, such as *C. meniscioides*, look much like some simply pinnate *Polybotrya* species (e.g., *P. serratifolia*) and are sometimes determined as such in herbaria. This resemblance in lamina cutting strongly suggests a near relationship of the two genera. In *Cyclodium*, species with a pinnate lamina, like *C. meniscioides*, have a subconform apical "pinna" (Fig. 7G) instead of a pinnatifid apex, and the lamina is thus imparipinnate. This particular lamina shape is often correlated with loss of dissection and can be seen in many unrelated genera, e.g., *Thelypteris*, *Cyathea*, *Cnemidaria*, and *Diplazium*. Those species of *Cyclodium* with the least divided laminae also show increased tendency toward dimorphism (e.g., *C. akawaiorum*, Fig. 6A; *C. calophyllum*, Fig. 7A, B). This may be in part related to the development of a hemiepiphytic habit, which is often correlated with dimorphy in many unrelated genera, e.g., *Lomariopsis*, *Polybotrya*, *Maxonia*, *Lomagramma*, and *Bolbitis*.

Venation.—On the basis of outgroup comparison, the general and primitive condition in *Cyclodium* is for the veins to be simply pinnate and unbranched in the ultimate segments. This pattern occurs in *C. inerme* (Fig. 11H), *C. guianense* (Fig. 9D), *C. rheophilum*, *C. trianae* (Fig. 13D, G), and *C. seemannii*. With loss of dissection and increased webbing, there is the almost inevitable consequence of anastomosing of veins, from weakly united (*C. heterodon* var. *abbreviatum*, Fig. 11F), to one or two pairs united at a more oblique angle (*C. varians*, Fig. 9K), to several pairs more strongly united between the costa and margin (*C. heterodon* var. *heterodon*, Fig. 11C; *C. calophyllum*, Fig. 7A, B; *C. akawaiorum*, Fig. 6C), to many pairs regularly united (veins meniscioid: *C. meniscioides*, Fig. 7G, I, K). These differences in venation were used to distinguish *Cyclodium* in the restricted sense, comprising one or two species. However, this character by itself is inadequate to delimit *Cyclodium*, as shown by the transitional venation types found in *C. calophyllum*, *C. heterodon*, and *C. varians*. Many genera of ferns contain both free-veined and reticulate-veined species, e.g., *Thelypteris*, *Polybotrya*, *Polypodium*, and *Pteris*, and in general this character is a poor one with which to circumscribe genera.

Vein endings in *Cyclodium* are not usually visible without partial clearing of the lamina, but in general they reach nearly to the margin. There is a marginal commissural vein in a few species, e.g., *C. akawaiorum* (Fig. 6C), a feature also found in *Polybotrya polybotryoides* (Baker) Christ and *Olfersia*. Vein endings in *Stigmatopteris* are usually easily visible (viewed from the adaxial surface), enlarged or clavate at the tip, and terminate well back of the margin.

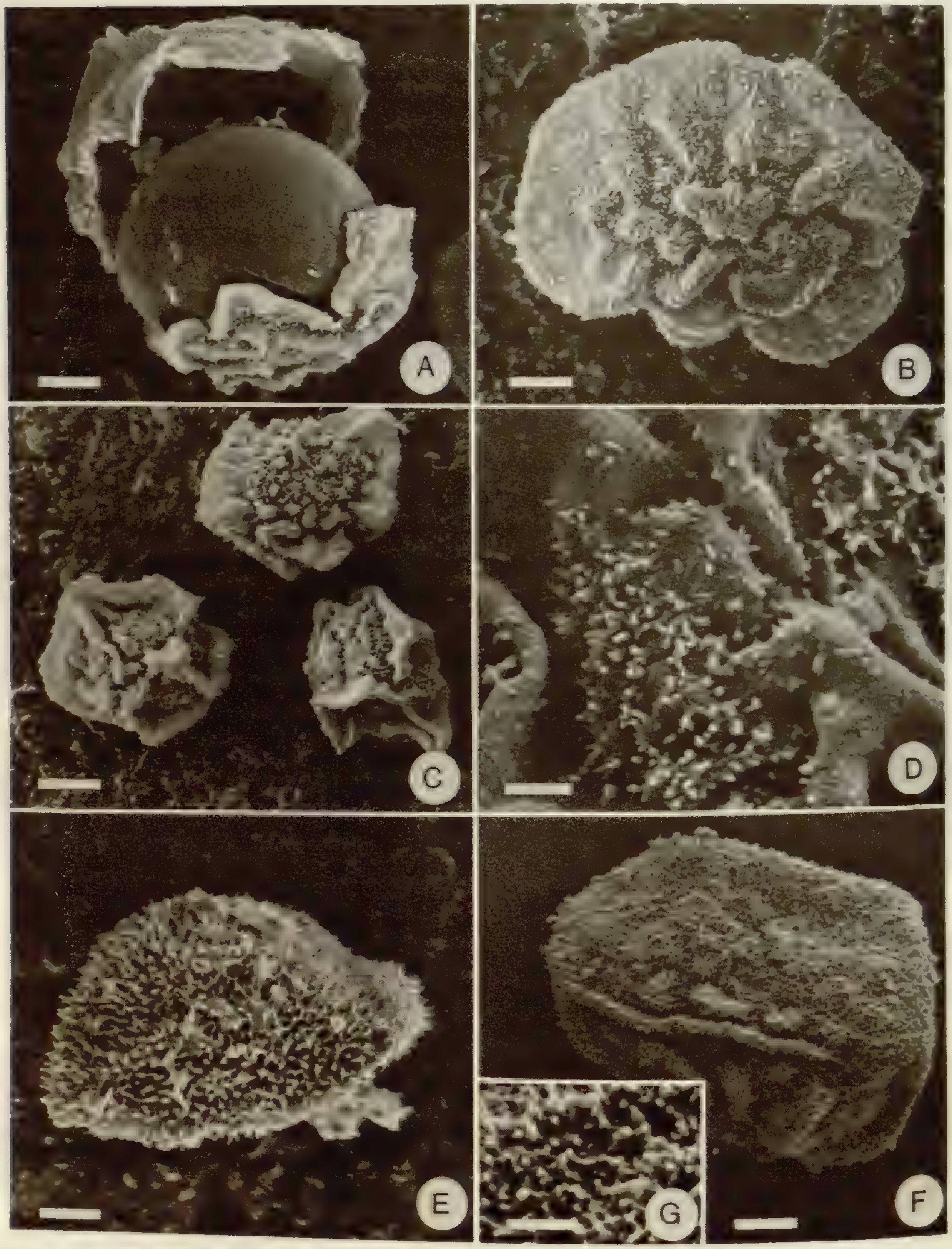


FIG. 2. Spores of *Cyclodium*. A, B. *C. guianense*, *Poole 1958*, NY; bar = 7.5 μm. C, D. *C. guianense*, *Davis 180*, GH; bar = 14.5 and 2.3 μm, respectively. E. *C. varians* hybrid, *Jenman s.n.*, NY; bar = 7.5 μm. F, G. *C. rheophilum*, *de Granville 2586*, F; bar = 7.5 and 1.9 μm, respectively.

Indument.—*Cyclodium* species are devoid of acicular hairs (as *Thelypteris* has), but bear short, spreading, blunt, yellowish or hyaline hairs 0.05–0.2(0.3) mm long. These hairs are most obvious in the grooves of the rachis and costae adaxially and at the base of the costae abaxially. Such hairs are most numerous and

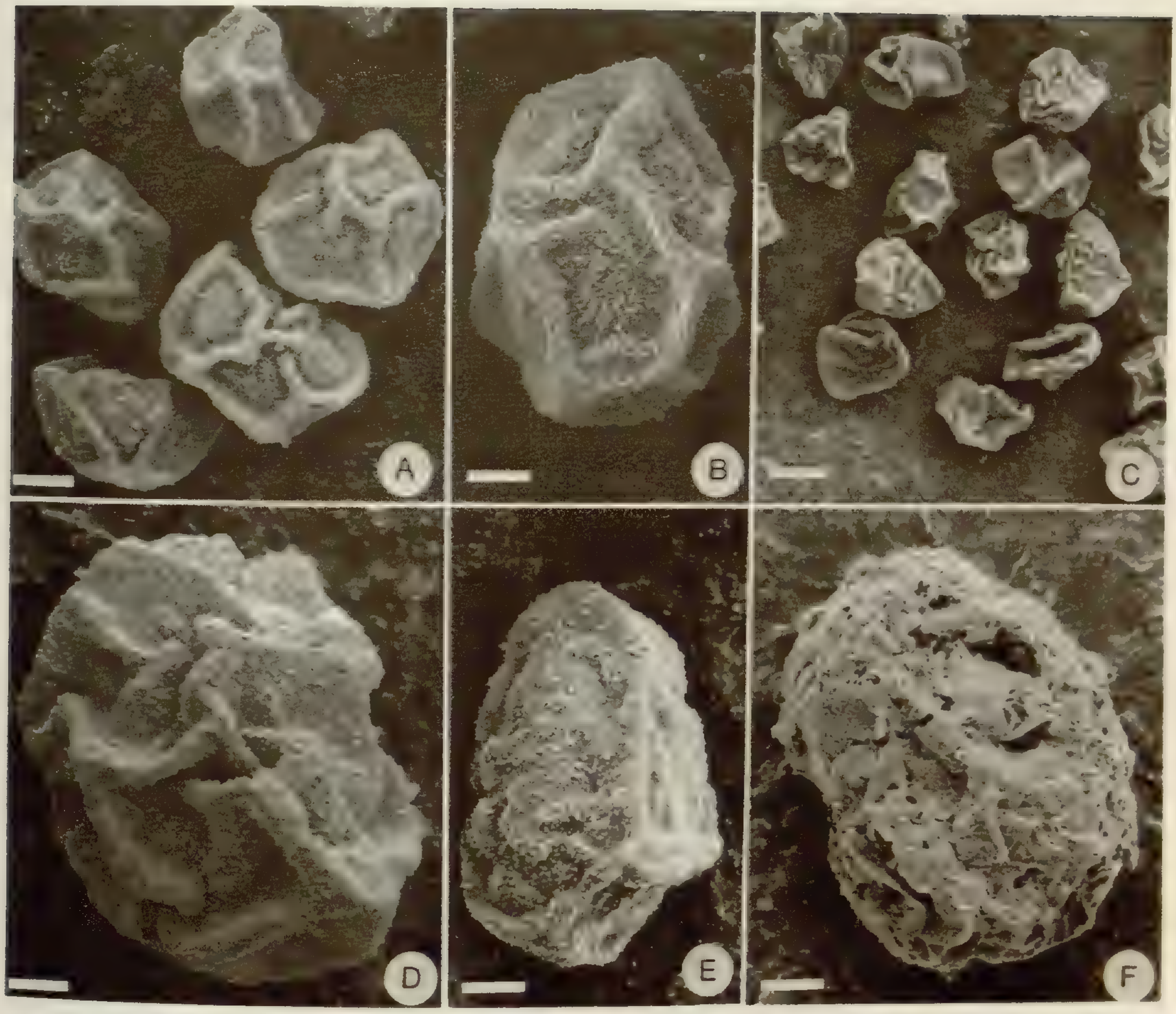


FIG. 3. Spores of *Cyclodium*. A, B. *C. inerme*, *Tillett et al. 44942*, NY; bar = 14.7 and 7.6 μm, respectively. C. *C. inerme*, *Maas & Westra 3990*, U; bar = 29 μm. D. *C. trianae* var. *chocoense*, *Killip & Cuatrecasas 38913*, US; bar = 7.7 μm. E. *C. trianae* var. *trianae*, *Killip & Smith 28540*, US; bar = 7.4 μm. F. *C. heterodon* var. *abbreviatum*, *Neowied s.n.*, BR; bar = 19.4 μm.

longest in *C. trianae* and *C. seemannii*, but at least a few can usually be found in all species. Scattered, uniseriate (basally 2 or 3 cells wide in some species), brownish, appressed, hairlike scales occur on the costae abaxially in most species. Very similar hairs (both the spreading hyaline type found on axes and the brownish appressed hairlike scales) occur in some species of *Polybotrya*, but are lacking or are not readily apparent in *Stigmatopteris*. *Cyclodium guianense* has broader, lanceolate, lighter colored scales on the costae abaxially (Fig. 9F).

Most *Cyclodium* species lack glands, but *C. seemannii* bears yellowish, resinous, sessile glands on the lamina abaxially (Fig. 13B). A few specimens of *C. guianense* and *C. trianae* have similar glands (Fig. 13E). All species have scattered, minute (0.1–0.3 mm), appressed, septate, reddish or brownish hairs (also reduced scales?) on the leaf tissue and veins abaxially.

Indusia.—With the exception of *C. trianae* and *C. seemannii*, which have orbicular-reniform indusia with a narrow sinus (Fig. 13B, D), *Cyclodium* species have peltate indusia (Figs. 7D, H; 9E; 11I). It seems likely that the peltate con-

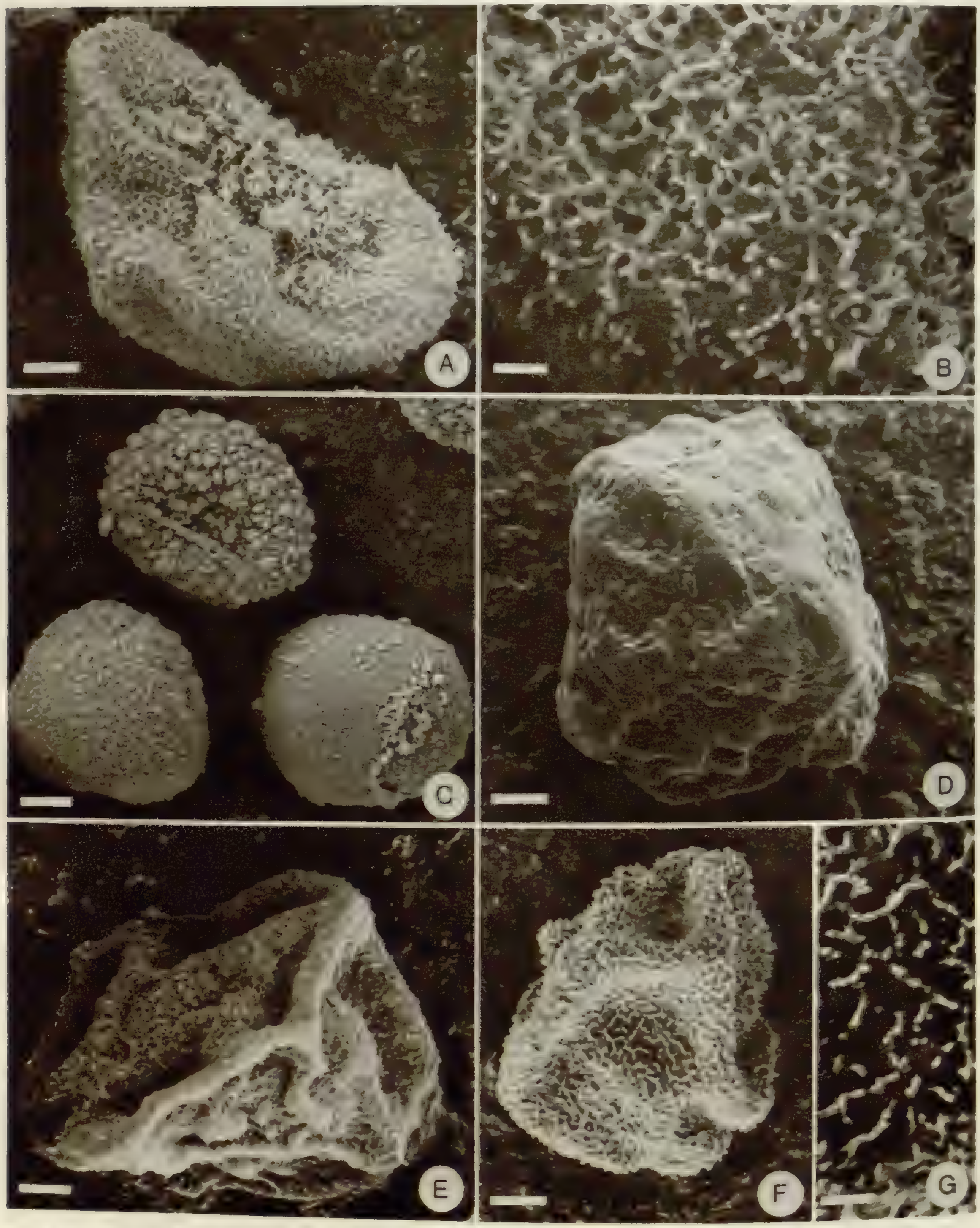


FIG. 4. Spores of *Cyclodium*. A, B. *C. meniscioides* var. *meniscioides*, *Lleras et al. P17441*, U; bar = 7.6 and 2.0 μm, respectively. C. *C. meniscioides* var. *meniscioides*, *Schulz 8695*, U; bar = 10.9 μm. D. *C. akawaiorum*, *K.E.R. 167*, pt, UC; bar = 14.7 μm. E. *C. meniscioides* var. *rigidissimum*, *K.E.R. 167*, pt, UC; bar = 7.5 μm. F, G. *Polybotrya sorbifolia*, *Glaziou 15714*, C; bar = 7.5 and 2.3 μm, respectively.

dition is derived in the genus, and that orbicular-reniform indusia represent an ancestral condition. Both character states are known in other dryopteroid genera. Closest relatives *Polybotrya* and *Olfersia* are exindusiate, while *Maxonia* has orbicular-reniform indusia. More distant relatives are either exindusiate (*Stig-*

matopteris) or have orbicular-reniform indusia (*Polystichopsis, Dryopteris, Arachniodes*). In *Cyclodium*, indusia are nearly always caducous; consequently they may not be present on mature fronds and many herbarium specimens. This feature distinguishes the genus from many other dryopteroid genera, where at least some indusia are retained even after spores are shed (as in *Dryopteris*).

Spores

Spores of *Cyclodium* are bilateral with a monolete scar. A variously folded and ornamented perispore surrounds a relatively smooth exine (Fig. 2A). The spores are mostly 44–65 μm long and 30–44 μm wide. The most common pattern is exemplified by spores of *C. inerme* (Fig. 3A, B), which has coarse rugae (inflated folds) with fine papillate surface projections. Similar spores occur in *C. trianae* var. *trianae* (Fig. 3D, E). A more intricate pattern of rugae occurs in *C. guianense* (Fig. 2, B–D). In *C. rheophilum*, the folds are less prominent, but the surface ornamentation is denser (Fig. 2F, G). Spores of *Cyclodium meniscioides* have a perispore with relatively few folds but bear a dense, anastomosing network of surface projections (Fig. 4A, B, E). There appears to be considerable variation in the surface pattern in this species (Fig. 4C), but this may be a function of spore maturity. Anomalous spores, without the usual pattern of ridges and surface ornamentation, were seen in *C. heterodon* (Fig. 3F) and *C. akawaiorum* (Fig. 4D). These spores were also considerably larger than usual, 100 μm long or more. Spores of a putative hybrid involving *C. varians* were mostly strongly malformed, but a few were densely echinate (Fig. 2E).

In some samples, a rather high proportion of the spores seemed collapsed (Fig. 3C). This is like the situation found in *Bolbitis* by Hennipman (1977), where presumably fertile and sexual species produce a high percentage of malformed spores.

In overall aspect, spores of *Cyclodium* are rather similar to spores of many other dryopteroid ferns (compare, e.g., illustrations of *Didymochlaena truncatula* (Swartz) J. Smith and *Cyclopeltis semicordata* (Swartz) J. Smith in Tryon & Tryon, 1982, pp. 518, 486). They are especially similar to spores of some *Polybotrya* species, e.g., *P. fractiserialis* (Baker) J. Smith (Tryon & Tryon, 1982, p. 541) and *P. caudata* Kunze (Moran, ms. in prep.). They also greatly resemble spores of *Olfersia cervina* (L.) Kunze (Tryon & Tryon, 1982, p. 541, as *Polybotrya cervina*). *Stigmatopteris nothochlaena* (Maxon) C. Chr. [=*S. jamaicensis* (Desv.) Proctor] (Tryon & Tryon, 1982, p. 522) has similar spores, but they lack the fine surface ornamentation of most *Cyclodium* species. Other *Stigmatopteris* species, e.g., *S. prionites* (Kunze) C. Chr. and *S. contracta* (Christ) C. Chr., seem also to lack fine surface ornamentation (A. Tryon, ms. in prep.). There is somewhat less resemblance to spores of *Polybotrya sorbifolia* Mett. ex Kuhn (Fig. 4F, G). In general, *Cyclodium* species lack the finely and densely echinate surface of many *Polybotrya* species (see, e.g., figs. 80.24, 80.28, 80.29 in Tryon & Tryon, 1982; Moran, ms. in prep.). Because of parallelism, convergence, and the extent of variation within genera, it seems doubtful that spore surface morphology alone can be used to characterize genera of dryopteroid ferns.

Chromosome Number

Only two previous counts, both for *C. meniscioides*, have been made in *Cyclodium*. Walker (1973) reported n = ca. 42 for a plant from Guyana [*Evemy* (=*Every*) & *Burgess 141*, cultivated at Kew], while Silva Araujo (1976) reported $2n = 60$ for a plant from Manaus, Amazonas, Brazil (*Araujo 244*, INPA). The latter is most likely an erroneous count or a misidentification; I have not seen the voucher.

I have obtained additional counts on cultivated material of *C. meniscioides* that establish the base number for this genus as $x = 41$. *Every & Burgess 57* (Kew accession no. 681-69-06186), K!, from Mataruma, Kirikava, Guyana, and *Every & Burgess 258* (063-70-00585), K!, from Essequibo, Guyana, showed unequivocally $2n = 41$ II at meiotic metaphase. Similarly, *Leppard 1278* (479-74-04441), K, from Brazil, showed $2n = 41$ II.

The closest relative, *Polybotrya*, *Olfersia*, and *Maxonia*, have all been reported as $n = 41$ (Walker, 1966), so this number in *Cyclodium* is not surprising. *Stigmatopteris* s.s. also has the same base number (Walker, 1966), as do nearly all dryopteroid ferns.

Hybrids

Several herbarium specimens suggest that hybridization occasionally takes place in *Cyclodium*. The primary area of sympatry, and thus the most likely area for hybridization to occur, is in the Guianas. *Jenman s.n.* (NY), Guyana, Pomeroon River, combines features of several species, particularly *C. varians*, *C. meniscioides*, and *C. inerme*. The long-creeping rhizome suggests that it is a hemiepiphyte, and the pinna lobing is very irregular. A few spores (Fig. 2E) are seemingly well-formed, but most are decidedly irregular. Several collections from Malali, Guyana (*Jenman s.n.*, NY) are also suggestive of hybridization. *De la Cruz 2880* (F, GH, NY, UC), from Kamakusa, Guyana, is also very irregular in pinna lobing and may represent a hybrid between *C. meniscioides* and *C. varians*. A collection from Pará, Brazil (*Spruce 26**, K) appears intermediate between *C. inerme* and *C. meniscioides*. Another collection from Pernambuco, Brazil (*Gardner 1218*, K), appears intermediate between *C. heterodon* and *C. meniscioides*.

All of these putative hybrids are apparently rare and are thus poorly known; all but one lack mature spores. Little more can be said about their origin and parentage until apparently hybrid plants can be studied in the field or until cytological materials can be obtained.

Ecology and Distribution

Species of *Cyclodium* occur in relatively undisturbed lowland and lower montane rainforests in the American tropics, from Trinidad and Panama throughout northern South America to Amazonian Bolivia, northern Argentina, and Paraguay. The preferred habitat, as judged from herbarium labels, appears to be in

rain forests and gallery forests, along streams, and, in the case of at least *C. meniscioides*, in waterlogged soils and swamps.

Most *Cyclodium* species occur between 0 and 800 m elevation. Exceptionally, *C. guianense*, *C. meniscioides* var. *meniscioides* and *C. trianae* var. *chocoense* are found at elevations up to 1450 m. The only species regularly known from middle elevations is *C. akawaiorum*, all collections of which have been made from 1000 to 1500 m.

The center of diversity is the Guianas, where six of the ten species occur; three others are centered in western Venezuela, Colombia, and Panama; an additional species occurs in coastal Brazil. The most wide-ranging species, *C. meniscioides*, occupies a range nearly coextensive with that of the genus; it is absent only from Panama and western Colombia. Five species (*C. akawaiorum*, *calophyllum*, *rheophilum*, *seemannii*, and *varians*) and several varieties have very restricted ranges and are represented by relatively few collections in herbaria. A few species, particularly *C. inerme*, *C. guianense*, *C. meniscioides*, and *C. trianae* var. *chocoense*, seem to be common understory ferns in portions of their ranges; in view of the widespread nature of many neotropical lowland ferns, it is a little surprising that these cyclodiums do not range into Central America, the Antilles, and many parts of South America.

Although most species of *Cyclodium* are terrestrial, several show a tendency to hemiepiphytism. The most notable in this regard is *C. akawaiorum*, which has been recorded as climbing five meters into the trees. Many collections of *C. meniscioides* have been noted as "epiphytes" on herbarium labels, but in general this species does not appear to climb more than one or two meters up trunks; it and other species of the genus are apparently always rooted in the ground. Data on *C. varians* is meager, but it probably also climbs trees in certain situations. One species, *C. rheophilum*, is a probable rheophyte; others, as *C. guianense*, may occasionally occur on rocks, but probably do not grow in stream beds. The remaining species are known as occurring only terrestrially.

Relationships

Interspecific.—Relationships within *Cyclodium* can be deduced from overall morphological similarities and differences (Fig. 5). Clearly, *C. inerme* is most closely related to a trio of species, *C. guianense*, *C. heterodon* var. *abbreviatum*, and *C. trianae* var. *chocoense*; characters indicating these affinities are the free venation and the pinnate-pinnatifid laminae. The last two taxa are allopatric with respect to *C. inerme*, and it seems likely that ecogeographic speciation has occurred. Varieties *abbreviatum* and *chocoense* appear to intergrade with var. *heterodon* and var. *trianae* respectively. Variety *trianae* is that member of the genus that most closely resembles *Polybotrya* and can serve as a possible evolutionary link to that genus; it is also closely related to the highly local and rare *Cyclodium seemannii*. The origin of *C. meniscioides*, with highly anastomosing venation and subentire pinnae as probable derived character states, is uncertain, but it seems most closely related to and possibly derived from *C. heterodon* var. *heterodon*. The highly local *C. akawaiorum* may be derived from *C. menis-*

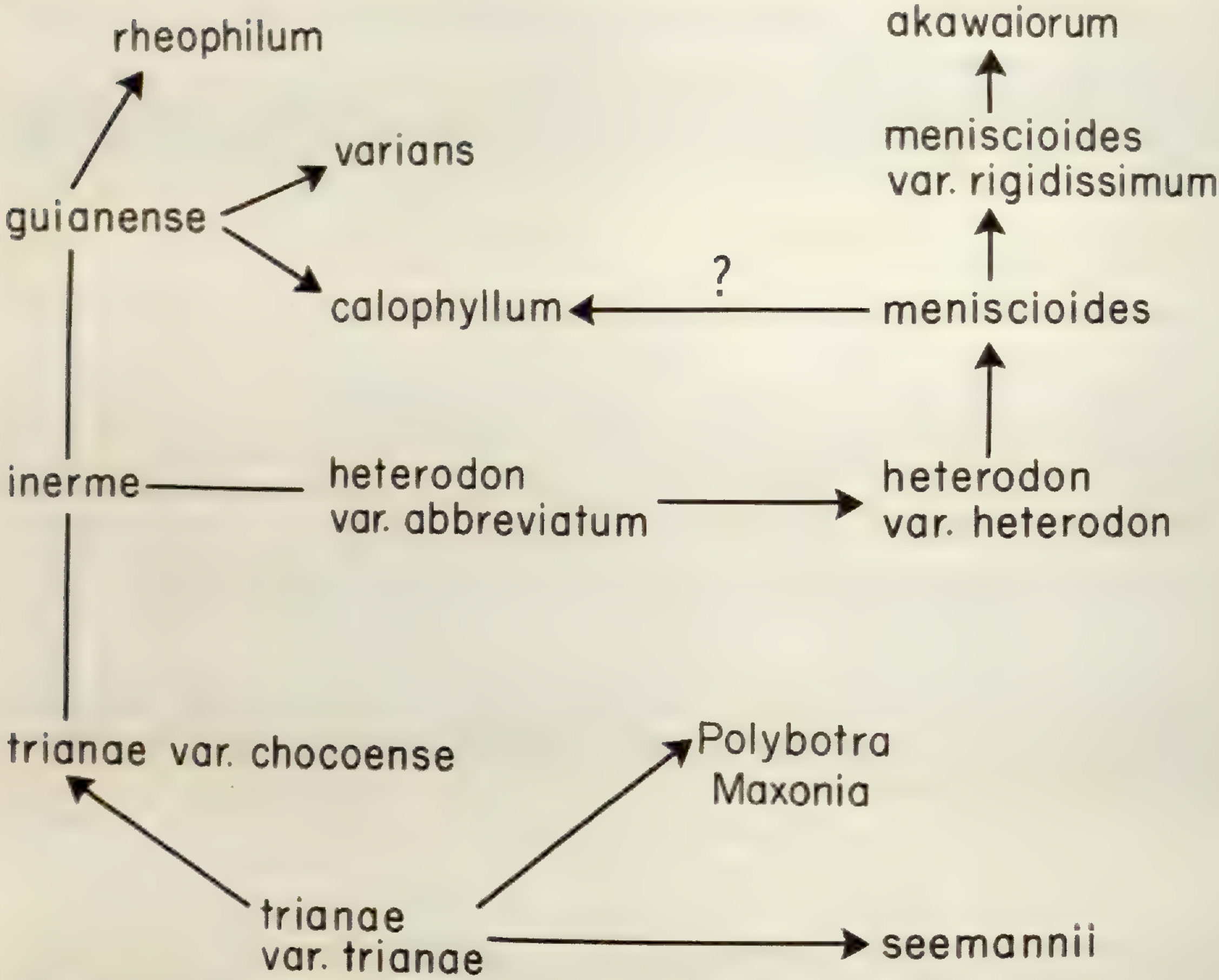


FIG. 5. Hypothetical relationships in *Cyclodium*. Arrows indicate direction of evolution.

cioides var. *rigidissimum*, but also shows some resemblance to *C. varians*. *Cyclodium varians* and *C. calophyllum* are both very restricted in range and show intermediacy in several characters between more wide-ranging species; it seems possible that they are relatively recent hybrid derivatives. Further investigation of this hypothesis is desirable. *Cyclodium rheophilum* is most closely related to and probably derived from *C. guianense* through ecological specialization.

The rooting of a phylogenetic tree depicting the relationships in Fig. 5 is uncertain, but it seems most likely that *C. trianae* var. *trianae* and *C. seemannii* represent the most primitive elements in the genus. Character states that have been used to arrive at this hypothesis are indicated in Table 1. In Fig. 5, I have indicated evolutionary direction only where morphological specializations provide good evidence.

Suprageneric.—The affinities of *Cyclodium* are without doubt with other dryopteroid genera. Characters uniting this assemblage are the usual presence of round-reniform or peltate indusia; bilateral spores with a variously winged perispore; pinnate or often more dissected lamina; midribs of penultimate segments grooved, with the rachis-groove usually open to admit the pinna-groove; numerous vascular bundles in the stipe bases; dictyostelic, scaly rhizome; and $x = 41$.

More specifically, *Cyclodium* is most closely allied to *Polybotrya*, differing from that in the presence of indusiate sori, the sporangia always in discrete,

TABLE 1. Character State Polarities in *Cyclodium*.

	Primitive	Derived
Habit	Terrestrial	Hemiepiphytic, rheophytic
Dissection	Decompound	Pinnate-pinnatifid, 1-pinnate
Apex	Pinnatifid	Conform
Veins	Free	Anastomosing
Areoles	1 or 2 series	>2 series
Submarginal vein	Absent	Present
Laminar glands	Absent	Present
Indusia	Sinus present	Peltate

round sori (never acrostichoid); monomorphic or weakly dimorphic fronds; lack of a strong hemiepiphytic habit; rhizome lacking black sclerchymatous sheaths surrounding the meristeles; and in the spores not densely and sharply echinate. The monotypic genus *Maxonia* is also closely related but differs in being strongly dimorphic, having a more dissected sterile lamina (to 3-pinnate-pinnatifid), possessing a dorsiventral rhizome, and having densely echinate spores. *Olfersia*, also monotypic, differs in having parallel venation with vein tips united by an intramarginal vein, a conform apical "pinna," strongly dimorphic fronds, acrostichoid fertile pinnae, and the absence of indusia; rhizome and stipe base scales are also lighter in color.

Stigmatopteris, with which *Cyclodium* has traditionally been combined or associated, is a more distant relative. It differs from *Cyclodium* primarily in being exindusiate, having pellucid-punctate glands on the lamina (most easily seen with transmitted light), pinnae with serrate apices, generally thinner laminae, lighter brown, thin, ovate-lanceolate scales on the stipe base and costae abaxially, and in having veins ending in a clavate tip well back from the margin (as seen on the adaxial side of lamina). *Stigmatopteris* species are always terrestrial or epipetric and have a short-creeping or suberect rhizome. Some *Cyclodium* species have been thought to possess punctate laminar glands (e.g., by Christensen, 1913, p. 74), but I have been unable to verify this. Sometimes the stomata (guard cells) appear lighter green than the surrounding lamina when viewed at about 30 times magnification and could easily be confused with pellucid dots of the kind seen in the lamina of *Stigmatopteris*.

Dryopteroid genera with which *Cyclodium* has a more remote relationship include *Phanerophlebia*, *Dryopteris*, *Arachniodes*, and *Polystichopsis*. The first of these differs from *Cyclodium* in having spinulose margins, 1-pinnate fronds, and the absence of minute hairs in the costal grooves. *Dryopteris* usually has a catadromic lamina distal to the proximal pinnae, reniform or round-reniform indusia, a suberect rhizome, and more ovate, lighter-colored stipe base scales. *Arachniodes* usually has a much more dissected lamina and round-reniform indusia. One species generally placed in *Arachniodes*, *A. macrostegia* (Hook.) Tryon & Conant, may have affinity to *Cyclodium*, particularly to *C. trianae*. It agrees with *Cyclodium* in having an anadromous lamina, a somewhat creeping rhizome, and in being found at low elevations, but differs in having a more dissected lamina, round-reniform indusia, and in lacking minute costal hairs

adaxially. *Polystichopsis* differs in having round-reniform indusia (or is exindusiate), axes densely long-pilose (some hairs acicular), generally thinner laminae, broader, more flaccid stipe base and rhizome scales, and ultimate segments that are usually mucronate at the tip. One species, *P. ochropteroides* (Baker) Morton, has a large greatly dissected lamina (4-pinnate) and approaches *Arachniodes macrostegia* in many characteristics. It was synonymized with that species by Tryon and Tryon (1982, p. 501) and also by Proctor (1985), but differs in the long-pilose axes and occurrence at higher elevations.

In assessing the relationship of *Cyclodium*, two New World tectarioid genera (for characterization of this group, see Holttum, 1984, and references cited therein), *Ctenitis* and *Lastreopsis*, have also been considered as possible allies but rejected on the basis of several characters: a lamina in which the axes of the penultimate segments are not grooved adaxially (often slightly raised), with ctenitoid hairs (short, multicellular hairs lacking an acicular tip, often with reddish cross walls, the cells collapsing upon drying) on this axis.

Several relatives of *Cyclodium* show a tendency toward anastomosing veins; *Polybotrya*, *Stigmatopteris*, and *Phanerophlebia* all have some members with this condition. Only *Phanerophlebia* agrees with most cyclodiums in having peltate indusia. Several genera are similar to *Cyclodium* in being primarily (or primitively) anadromous: *Polybotrya*, *Maxonia*, *Polystichopsis*, and at least some *Arachniodes*. Strong dimorphism is found in *Polybotrya*, *Maxonia*, and *Olfersia*, but several cyclodiums show a tendency toward dimorphism. It seems likely that the common tendencies shown by various dryopteroid genera are a reflection of common ancestry and perhaps even reticulate evolution. These shared characters make generic delimitation in this group especially difficult.

Taxonomic Treatment

Cyclodium Presl, Tent. Pterid. 85. 1836.—*Aspidium* sect. *Cyclodium* (Presl) Hooker, Sp. Fil. 4:36. 1862.—Lectotype (chosen by J. Smith, Hist. Fil. 203. 1875): *Aspidium confertum* Kaulf. [= *Cyclodium meniscioides* (Willd.) Presl var. *meniscioides*]. Hooker referred to infrageneric taxa (denoted by the symbol §) in *Aspidium* as subgenera or sections (p. 5, 6), but called *Aspidium* § *Cyrtomium* (p. 37) a section; hence I adopt sectional rank for his *Aspidium* § *Cyclodium*. The type of *Cyclodium* has generally been considered to be *Aspidium meniscioides* (Christensen, Ind. Fil. xxv. 1906; Copeland, 1947; Index Nominum Genericorum 1:470. 1979; Tryon & Tryon, 1982, p. 519); however, John Smith (1875) selected *A. confertum*, one of the original species, and I see no reason to reject his choice.

Dryopteris subg. *Stigmatopteris* (C. Chr.) C. Chr. group *Peltochlaena* Fée ex C. Chr., Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Afd. VII. 10: 74. 1913.—Lectotype (chosen here): *Dryopteris subobliquata* (Hook.) Kuntze [=*Cyclodium inerme* (Fée) A. R. Smith. The other syntypes are *Dryopteris varians* (Fée) Kuntze and *D. sancti-gabrieli* (Hook.) Kuntze.

Peltochlaena Fée, Gen. Fil. (Mém. Fam. Foug. 5) 289. 1852. nom. provis. et illegit.—TYPE: "Peltochlaena nephrodiiformis Fée." Fée clearly adopted this as a provisional name ("Nous indiquons parmi les cyclodiées . . . un genre que nous n'osons pas constituer définitivement, la description que nous donnons de l'espèce type, la seule que nous connaissions, le fera suffisamment connaître à titre provisoire."); thus it has no taxonomic standing [ICBN, Art. 34.1(b)]. (Pichi Sermolli, Webbia 32:351. 1978).

Plants terrestrial or hemiepiphytic; rhizome dictyostelic, creeping, clothed at and near apex with lanceolate or linear-lanceolate, usually castaneous scales, these entire or denticulate; trophopods lacking; stipes non-articulate, with 8–16 vascular bundles arranged in a ring; laminae monomorphous to often slightly or decidedly dimorphous (the fertile fronds more erect, longer-stiped, and with contracted fertile segments), usually pinnate to 2-pinnate-pinnatifid (rarely simple), anadromous with the segments or pinnules on the acroscopic side of a pinna generally longer and arising first (if simply pinnate, then an acroscopic lateral vein arising first); laminae gradually reduced to a pinnatifid apex or imparipinnate; rachis and costae adaxially grooved, the grooves ± confluent from one axis to the next; grooves adaxially and sometimes rachis and costae abaxially with uniformly short, blunt-tipped hairs 0.05–0.2(0.3) mm long; costae abaxially with uniseriate (basally to 3 cells wide in a few species), brownish or reddish-brown, appressed hairs (actually reduced scales); veins free and the basal pair of a group meeting the margin at or near the sinus, or connivent below the sinus, or regularly anastomosing (uniting in pairs) to form a series of areoles, these united veins often producing an excurrent veinlet; lamina chartaceous to subcoriaceous or coriaceous, not translucent, lacking pellucid-punctate glands; indusia peltate (8 spp.) or round-reniform (2 spp.), caducous often before the sporangia mature, glabrous or glandular-ciliate on the margin and sometimes the surface; sporangia long-stalked, the stalk 0.4–0.5 mm long, capsules often abscissing at the tip of the stalk; spores bilateral, monolete, with a folded, winglike perispore; $x = 41$.

KEY TO SPECIES AND VARIETIES

1. Veins regularly anastomosing; laminae monomorphous to strongly dimorphous; indusia peltate.
2. Laminae with a ± conform, entire or crenate apical segment.
3. Lateral pinnae ovate-lanceolate, up to 11 cm long and 2.3 cm wide; costae abaxially lacking scales; indusial margin entire; areoles 1- or 2-seriate between costa and margin. ..... 1. *C. akawaiorum*
3. Lateral pinnae elliptic, 10–25 cm long, (2)4–8 cm wide; costae abaxially with narrow scales; indusial margin usually ciliate; areoles 3–7-seriate.
4. Pinnae serrulate; sterile pinnae to 11 cm long, 3 cm wide ..... 6b. *C. meniscioides* var. *paludosum*
4. Pinnae entire or crenate; sterile pinnae usually larger.

5. Veins strongly raised on adaxial surface; pinnae of sterile fronds approximate and overlapping ..... 6c. *C. meniscioides* var. *rigidissimum*
5. Veins not strongly raised on adaxial surface; pinnae distant, not overlapping ..... 6a. *C. meniscioides* var. *meniscioides*

2. Laminae with a confluent, pinnatifid apical segment.
6. Costae and rachis abaxially persistently scaly ..... 2. *C. calophyllum*
6. Costae and rachis abaxially lacking scales.
7. Rhizome long-creeping (sometimes hemiepiphytic?), with dark purplish-brown linear scales; laminae subdimorphous, the fertile with pinnae 0.8–1.5 cm wide; (Guyana, Trinidad) ..... 10. *C. varians*
7. Rhizome short-creeping (plants terrestrial), with brownish, linear-lanceolate scales; laminae monomorphous, pinnae usually more than 2 cm wide.
8. United veins 2 or 3 pairs; pinnae sinuate ..... 4a. *C. heterodon* var. *heterodon*
8. United veins 1 pair; pinnae pinnatifid, with a cuneate base ..... 4b. *C. heterodon* var. *abbreviatum*

1. Veins all free; laminae monomorphous; indusia peltate or orbicular-reniform.
9. Indusia peltate; laminae pinnate to pinnate-pinnatifid.
10. Pinnae linear-lanceolate, less than 1 cm wide, crenate; plants rheophytic; (French Guiana) ..... 7. *C. rheophilum*
10. Pinnae lanceolate, greater than 1 cm wide, crenately lobed to pinnatifid.
11. Scales several cells wide on costae abaxially; pinnae (15)20–30 pairs ..... 3. *C. guianense*
11. Scales lacking on costae abaxially; pinnae 9–15(24) pairs.
12. Rhizome scales 3–5(7) mm long, relatively inconspicuous; sori often impressed (lamina embossed adaxially) ..... 5. *C. inerme*
12. Rhizome scales ca. 1 cm long, often conspicuous; sori not or only slightly impressed ..... 4b. *C. heterodon* var. *abbreviatum*

9. Indusia orbicular-reniform, with a narrow sinus; laminae pinnate-pinnatifid to bipinnate-pinnatifid.
13. Lamina abaxially with yellowish to orangish, sessile, globose glands ..... 8. *C. seemannii*
13. Lamina lacking such glands.
14. Laminae bipinnate throughout most of their length, largest pinnules pinnatifid, constricted at their base and short-stalked ..... 9a. *C. trianae* var. *trianae*
14. Laminae shallowly to usually deeply pinnate-pinnatifid, if bipinnate, the pinnules entire to crenate, adnate and decurrent ..... 9b. *C. trianae* var. *chocoense*

1. **Cyclodium akawaiorum** A. R. Smith, sp. nov. (Fig. 6).—TYPE: Guyana, NW-facing slopes of Mt. Roraima, vicinity of Camp 6 near end of Waruma Trail, ca. 1 mi N of the prow, 5°16′30″N, 60°44′45″W, 4200–4300 ft, *Warrington et al. K.E.R. 76* (UC!; isotype K, not seen). From this same population, plants were grown at Kew under the number K.E.R. 1207 (Kew Accession No. 174-78-01565, K!; 178-78-15565, UC!).

Cyclodio meniscioide var. *rigidissimo* affinis, a qua imprimis differt habitu hemiepiphytico, frondibus parvioribus paribus paucioribus venarum anastomosantium, pinnis sterilibus paucioribus non imbricantibus, et costis epaleatis.

Plants hemiepiphytic, climbing up to 5 m into trees; rhizomes long-creeping, up to 3+ cm between stipe bases, ca. 6–8 mm diam., densely clothed with lanceolate scales at and up to 25 cm behind the apex, the scales up to ca. 1.0 cm long, 1.2 mm wide, castaneous and sometimes with a darker midstripe, strongly erose-denticulate, the teeth composed of several cells; fronds dimorphic, the fertile much narrower but slightly exceeding the sterile in length, up to 60 cm long, 6 cm wide, the sterile up to 50 cm long, 12–18 cm wide; stipe one-half to nearly equalling the lamina in length, 1.5–3.0 mm diam., stramineous to brownish, epilose, with scattered scales like those of rhizome but these more strongly bicolorous and the margin more strongly erose-dentate, sometimes even fimbriate; sterile laminae up to 35 cm long, 18 cm wide, with 6–14 pairs of lateral pinnae, these ± gradually reduced distally, with a free lanceolate terminal segment that is about as large as, or slightly larger than, the largest pinnae; fertile laminae up to 25 cm long, with up to 9 pairs of lateral pinnae and a subsimilar terminal segment; sterile pinnae ovate-lanceolate, up to 10.5 cm long, 2.3 cm wide, the lowermost stalked to 5 mm, the stalk adaxially arching, the pinnae ± rounded at base, acute at tip, entire or sinuate; fertile pinnae elliptic, up to 4 cm long, 0.8 cm wide, obtuse at tip, entire; veins on both kinds of fronds anastomosing to form 1 or 2 rows of areoles between costa and margin, the ultimate vein endings forming a submarginal commissural vein, veins often strongly raised adaxially; costae and costules abaxially with scattered, ± appressed, reddish-brown, minutely septate hairs up to ca. 0.5 mm long, lacking scales or glands; translucent light yellowish or whitish glandular hairs 0.1 mm long mostly confined to costal and rachis grooves and pinna bases; sori in (1)2 rows between costa and margin, discrete or subconfluent with age, with large, reddish-brown, entire, peltate indusia 1.0–2.0 mm diam.

Paratypes: GUYANA. Mt. Roraima, Paikwa Trail, 4600 ft. [actually 4300 ft, P. Edwards, in litt.], 28 Oct 1973, *Persaud 110* (NY! K, BRG, not seen). VENEZUELA (4 coll. seen). **Bolivar** (4). Salto La Danta, 1250 m, *Ruiz-Terán & López-Palacios 11473* (NY); Cerro La Danta, NW of Cerro Venamo, 1040–1060 m, *Steyermark & Nilsson 9* (US, VEN); Cerro Venamo, NW slopes, 1100–1300 m, *Steyermark & Nilsson 124* (NY, US, VEN); Cerro Venamo, SW slopes, 1400–1450 m, *Steyermark et al. 92644* (GH, NY).

Discussion.—This species is most closely related to *C. meniscioides*, especially var. *rigidissimum*, differing from that in the smaller fronds with fewer pairs of anastomosing veins, non-overlapping sterile pinnae, lack of costal scales, and fewer pinna pairs. Fertile and sterile pinnae are entire or only faintly sinuate in

FIG. 6. *Cyclodium akawaiorum*, *Persaud 110*, NY. A. Habit. B. Juvenile frond. C. Sterile pinna. D. Sori and indusia. E. Scale of rhizome apex.

C. akawaiorum. Those few collections in herbaria are often identified as *Polybotrya*, probably because of the climbing rhizome and general similarity to some simply pinnate *Polybotrya* species. Most collections seen lack fertile fronds.

Although there is as yet inadequate information concerning several species of *Cyclodium*, *C. akawaiorum* is apparently the only species in the genus that is a high-climbing hemiepiphyte; *C. meniscioides* is sometimes a low-climbing hemiepiphyte, as may be *C. varians*. Notes with the type collection indicate that plants were common but mostly sterile, growing "from bases of bryophyte covered trees, under trees amongst buttress roots, and also on some palm stems, climbing to 15 ft., stems with conspicuous roots to only 6″ above ground level .... Sporelings and young plants common in thick humus on forest floor .... Stock soon becomes creeping, and climbs a convenient support." All Venezuelan collections were also noted as climbing or "epiphytic" by their collectors.

Peter Edwards (in litt.) indicates that the Persaud collection was probably made from the same population as K.E.R. 76. The species epithet honors the local Akawaio Indians who, according to Edwards, were essential in conducting the Kew Expedition to Roraima (K.E.R.); one individual in particular was the first to spot the new *Cyclodium*.

9874

2. **Cyclodium calophyllum** (Morton) A. R. Smith, comb. nov. (Fig. 7, A–D).—
9875 *Dryopteris calophylla* Morton, Bull. Torrey Bot. Club 66:49. 1939.—TYPE: Colombia, Dept. Santander, Magdalena Valley, vicinity of Barranca Bermeja, 100–500 m, *Haught 1353* (US, 3 sheets!; isotypes GH! MICH!).

Rhizomes creeping; fronds to ca. 1.5 m long, somewhat dimorphous, the sterile shorter and with wider pinnae than the fertile; stipes ca. ½–¾ the lamina length, stramineous to tan, short-pubescent with hairs ca. 0.2 mm long or less, also with scattered, persistent, linear-lanceolate scales to 6 mm long, these brown, denticulate; rachises with scales similar to those of stipes; laminae ca. 60–80 cm long, up to 30 cm wide, pinnate with (2)15–18 pairs of lateral pinnae, these subabruptly reduced distally to a shallowly pinnatifid apex; pinnae subentire, crenulate (sterile), or shallowly lobed (fertile) ¼ or less the distance to costa (lobes as wide as or wider than long), up to 25 cm long, the fertile 2.0–2.5 cm wide, the sterile 3.0–3.5 cm wide, lowermost stalked up to 10 mm, rounded to truncate at base, acute to acuminate at apex, ± equilateral or acroscopic side slightly wider and with a small auricle at base; basal anterior veinlets free, next 3 or 4 pairs of veinlets anastomosing to form 3–4 series of areoles between costa and margin, with (30)40–60 pairs of lateral veins per pinna, these 3.5–5.0 mm apart; costae (and less obviously costules) abaxially with numerous filiform to linear-lanceolate, light brown, twisted scales, these uniseriate at apex and leaving a reddish spot after dehiscence; laminae chartaceous to subcoriaceous; sori in 3–5 ± regular rows between costa and margin, discrete, with relatively large, brownish, peltate, glabrous, entire indusia.

Distribution.—Known only from Depts. Santander and Norte de Santander, Colombia, and Edo. Zulia, Venezuela (Fig. 8). All collections are from low elevations, 100–500 m.

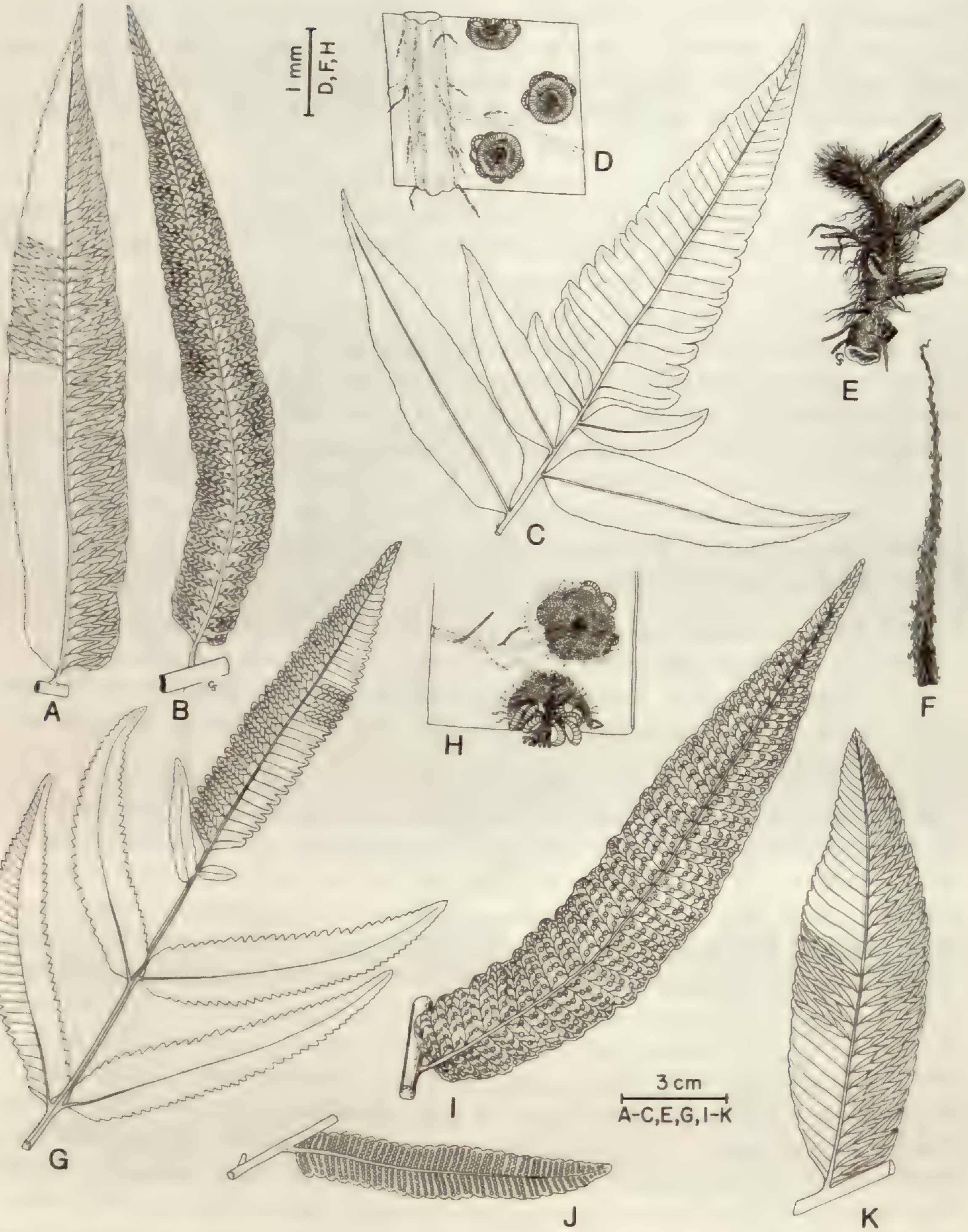


FIG. 7. *Cyclodium calophyllum* and *C. meniscioides*. A–D. *C. calophyllum*, *Haught 1353*, GH. A. Sterile pinna. B. Fertile pinna. C. Blade apex. D. Sori and indusia. E–K. *C. meniscioides* var. *meniscioides*. E and F. Rhizome and scale at apex, *Hitchcock 17124*, NY. G. Apex of fertile blade, *Prance et al. 59224*, GH. H and I. Sori, indusia, and fertile pinna, *de la Cruz 3119*, GH. J. Fertile pinna, *Hitchcock 17124*, NY. K. Sterile pinna, *Prance et al. 59224*, GH.

Discussion.—In nearly all of its characters, this species is intermediate between *C. meniscioides* and *C. guianense*, and it would not be surprising if it arose through allopolyploidy. However, the range of *C. calophyllum* appears to be in an area where neither of these possible parental species has been found.

FIG. 8. Distributions of *Cyclodium calophyllum*, *C. meniscioides* var. *meniscioides*, and *C. varians*.

It differs from *C. guianense* in having several pairs of anastomosing veins, subdimorphous laminae, larger fronds, and wider, more entire sterile pinnae. From *C. meniscioides* it is distinguished by having fewer pairs of anastomosing veins, not so strongly dimorphous laminae, fertile pinnae with discrete sori, a pinnatifid apical segment, and an eciliate indusial margin.

Little is known of the habitat of this species. The sole collection from Venezuela was recorded as growing along a slope at the edge of a stream.

Additional collections: COLOMBIA. **Norte de Santander**: Catatumbo, Campo Tibu, 200 m, *Bischler 2461* (G, 2 sheets; COL, not seen); Catatumbo, Puerto Barco, edge of Río Catatumbo, 150 m, *Bischler 2592* (G, 2 sheets). VENEZUELA. **Zulia**: 6 km ENE of Río de Oro, 100–350 m, *Liesner & González 13307* (MO, not seen; UC).

3. **Cyclodium guianense** (Klotzsch) A. R. Smith, comb. nov. (Fig. 9, C–F).—*Aspidium guianense* Klotzsch, Linnaea 20:364. 1847.—*Polystichum guianense* (Klotzsch) Presl, Epim. 58. 1849 [as "gujanense"].—*Aspidium abbreviatum* var. *guianense* (Klotzsch) Baker in Martius, Fl. Bras. 1(2):

FIG. 9. *Cyclodium guianense*, *C. rheophilum*, and *C. varians*. A and B. *C. rheophilum*, *de Granville 2586*, F. A. Habit. B. Larger pinna. C–F. *C. guianense*. C and D. Blade apex and largest pinna, *Maguire et al. 37391*, NY. E and F. Sori, indusia, and rhizome scale, *Schultes & Cabrera 13928*, US. G–K. *C. varians*, *Britton & Britton 2941*, US. G. Rhizome. H. Rhizome scale. I. Fertile pinna. J. Apex of sterile blade. K. Largest sterile pinna.

13977 464. 1870.—*Dryopteris guianensis* (Klotzsch) Posthumus, Ferns Surinam
13978 51. 1928.—*Stigmatopteris guianensis* (Klotzsch) C. Chr., Ind. Fil. Suppl. 3:174. 1934.—TYPE: Guyana, *Schomburgk 1157* (B?, not seen; isotypes K! UC!).

24910 *Polypodium sancti-gabrieli* Hook., Sp. Fil. 4:233. 1862.—*Nephrodium* 24909 *sancti-gabrieli* (Hook.) Baker in Martius, Fl. Bras. 1(2):469. 1870.—*Dryopteris*

sancti-gabrieli (Hook.) Kuntze, Rev. Gen. Pl. 2:813. 1891.—*Stigmatopteris sancti-gabrieli* (Hook.) C. Chr., Ind. Fil. Suppl. 3:175. 1934.—TYPE: Brazil, Amazonas, São Gabriel, *Spruce 2153* (K!; isotypes BM!-2 sheets, BR!-2 sheets, G! P!).

Plants terrestrial; rhizomes short-creeping (up to ca. 9 fronds per 3 cm.), 0.7–1.2 cm diam., at apex with castaneous or brown, lanceolate scales ca. 10 mm long, up to 1 mm wide, scales entire or erose-denticulate; fronds 80–150 cm long, monomorphous or subdimorphous, the fertile slightly taller than the sterile; stipe ½–⅔ the lamina length, tan to brown, 3–6 mm diam., with scattered scales; laminae up to 80 cm long, 20–40 cm wide, with (15)20–30 pairs of lateral pinnae, these gradually or subabruptly reduced to a confluent, pinnatifid apex; rachises often with scattered persistent scales up to 5 mm long, these somewhat bicolorous with a darker central stripe and lighter fimbriate margins; pinnae linear-lanceolate, (9)11–20(24) cm long, 1.3–2.5(3.7) cm wide, 2.5–5.0(7.5) cm distant, incised ⅕ (rarely to ½) or less the distance to costa, rounded at base or basiscopic side narrower and cuneate, acroscopic side often subauriculate, margins crenate or crenately incised for most of their length, serrate towards the apex; segments wider than long, rounded or blunt at tip, costules 3–5 mm distant; veinlets arising from costules, the anterior (1)2 veins and posterior 1(2) vein of a segment ending in lamina below the sinus, the remaining veinlets meeting margin above sinus, or veinlets of sterile pinnae all running toward margin; costae abaxially with scattered, appressed, amorphous, ovate, tan scales 0.5–3.0 mm long, up to 0.3 mm wide; lamina abaxially glabrous or with yellowish to reddish resinous glands on the surface, also often with scattered, reddish-brown, appressed hairs up to 1 mm long; sori 2–4(6) pairs per segment, discrete, impressed, with a large, tan, eciliate, entire or slightly erose, peltate indusium 1.0–1.5 mm diam.

Distribution.—Trinidad, Guyana, Surinam, French Guiana, northern Brazil (Amazonas, Pará, Roraima), Venezuela (Amazonas, Apure, Bolívar, Delta Amacuro, Táchira), and Colombia (Caquetá, Norte de Santander, Vaupés) (Fig. 10). A report by Christensen (1913) of a collection from Edo. Mérida, Venezuela (*Engel 231*, B, Sardoneta) may refer to a Colombian collection from Sardinata (Dept. Norte de Santander); Engel is known to have collected in both western Venezuela and eastern Colombia. Known from low elevations, 0–800(1200) m, mostly in primary lowland and lower montane rain forests.

Discussion.—*Cyclodium guianense* is related most closely to *C. inerme* and *C. calophyllum* (which see for a discussion of the differences). It appears to be much more common than *C. inerme* in the Amazonian basin, but much less common in Guyana; in Surinam and French Guiana both are well represented in herbaria, but *C. inerme* appears to be the most common.

Over most of its range, *C. guianense* appears relatively uniform. The most aberrant collection is the single one from Pará (*Prance & Pennington 1825*), which has the largest fronds and most deeply incised pinnae of all specimens seen. Nearly all collections of *C. guianense* are recorded as growing terrestrially, but *Smith 2745* from Guyana was noted as being an epiphyte in a swamp. Apparently it is never a hemiepiphyte.

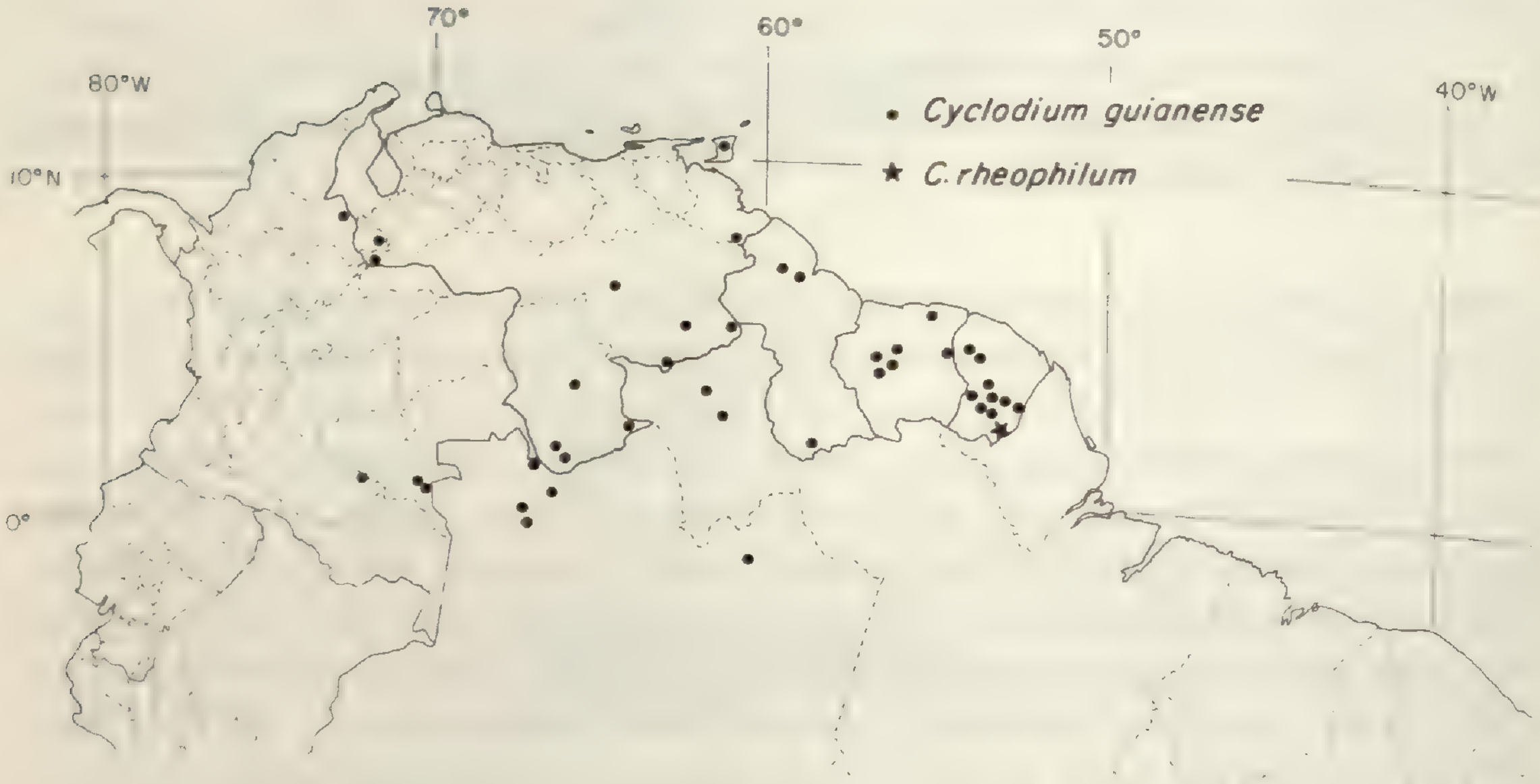


FIG. 10. Distributions of *Cyclodium guianense* and *C. rheophilum*.

Representative specimens: TRINIDAD (3 coll. seen). *Fendler 97* (GH, K, NY, UC, US); *Prestoe 1232* (NY), *s.n.* (BM). GUYANA (4). Imoti Creek, Waini River, *Beckett s.n.* (K, NY); Basin of Essequibo River, near mouth of Onoro Creek, *A. C. Smith 2745* (NY); region of Mt. Raywa, head of Pomeroon River, *Jenman s.n.* (K, NY). SURINAM (10). Emmaketen, *Daniëls & Jonker 744* (U), *852* (U); Bakhuis Mts., inter flum. Kabalebo et Coppename Sinistrum, *Florschütz & Maas 2975* (GH, U, US), *3047* (U); lower slopes of Frederik Top, 3.5 km SSE of Juliana Top, *Irwin et al. 54923* (MO, NY). FRENCH GUIANA (10). Sommet Tabulaire, ±45 km SE of Saül, *Cremers 6389* (Z); Sommet des Monts Atachi Bacca, *de Granville, C.123* (P, U); Rivière Petite Ouaqui, *de Granville 1860* (P, Z). BRAZIL (10). **Amazonas** (7): São Gabriel, Rio Negro, *Holt & Blake 602* (GH, NY, US); Cano Tucano, Rio Cauaburí, *Maguire et al. 60279* (NY); S slope of Morro Ximaio, along Rio Negro between Manaus and São Gabriel, *Poole 1922* (F, NY). **Pará** (1): vicinity of Cachoeira, Km. 96, Road BR 22, Capanema to Maranhão, *Prance & Pennington 1825* (F, MO, NY, U, US). **Terr. Roraima** (2): Upper slopes of Serra da Lua, *Prance et al. 9452* (MO, NY, U); Rio Uraricoeara, vicinity of Uaicá airstrip, *Prance et al. 19947* (G, NY). VENEZUELA (15). **Apure** (1): Dto. Paéz, between Cutufí on Río Cutufí and Río Sanare, *Davidse & González 21735* (MO). **Bolivar** (4): Cerca de boca Tirika Caroní, *Cardona 1641* (US); NE of Serranía Pia-soi, *Steyermark 90656* (GH, NY, US, VEN); Salto de Pará, Medio Caura, *Williams 11381* (F, US). **Táchira** (1): 10 km E of La Fundación, *Liesner & González 10375* (MO, UC). **Terr. Fed. Amazonas** (8): Alto Orinoco, Ugueto, *Croizat 782* (F, U, VEN); Cerro Duida, SE slopes along Caño Negro, *Steyermark 57958, 58077* (F, US); Cerro de la Neblina, Río Yatua, *Maguire et al. 36875* (NY, US), *36883* (NY), *37391* (F, NY, US). **Terr. Fed. Delta Amacuro** (1): E side of Río Cuyubini, Cerro La Paloma, *Steyermark 87654* (NY). COLOMBIA (7). **Caquetá** (2): Apaporis River, Cerro Castillo, *Schultes 5659* (GH, US). **Norte de Santander** (2): Catatumbo, Petrólea y alrededores, *Bischler 2628* (G). **Vaupés** (3): trail from house of Enrique Portua to Cerro Mitú, *Davis 180* (COL, GH, U); between Mitú and Javareté, Cerro Tipiaca, *Schultes & Cabrera 19329* (NY, US).

4. *Cyclodium heterodon*

Plants terrestrial; rhizomes short- to long-creeping, thick, 1.0–1.5 cm diam., at apex with castaneous, shining, linear-lanceolate, entire or minutely denticulate scales ca. 1.0 cm long, 0.5–1.0 mm wide; fronds 75–150 cm long, monomorphous or weakly dimorphous, with stipes ca. equalling the lamina length, stramineous to tan, 3–6 mm diam., with persistent scales similar to those of rhizome at base;

blade 50–75 cm long, 20–30 cm wide, with 12–16(22) pairs of lateral pinnae, these gradually to subabruptly reduced distally to a confluent shallowly to deeply pinnatifid apex; pinnae lanceolate to ovate-lanceolate, stalked to 1.5 cm, with a narrowly to broadly cuneate base, subentire or sinuate to broadly crenate or incised up to ⅘ their width (distal pinnae less incised than proximal ones); segments rounded to blunt at apex, often strongly oblique, 6–9 mm between adjacent costules; veinlets simple or often forked, arising from costules, connivent at or near the sinus, or more often connivent below the sinus with a common veinlet running to sinus, or basal pair united at an oblique angle below sinus with an excurrent veinlet, next 1 or 2 pairs alternately anastomosing with this excurrent veinlet; costae abaxially with scattered, appressed, reddish-brown, septate hairs (actually uniseriate scales) to 1.0 mm long, also usually with a few scattered linear tan scales 2 or 3 cells wide at base; sori 6–8(12) pairs per segment, discrete, not or only slightly impressed, with a large, peltate, tan, glabrous indusium 1.0–1.6 mm wide.

4a. *Cyclodium heterodon* var. *heterodon* (Fig. 11, A–C)

Cyclodium heterodon (Schrader) Moore, Ind. Fil. 275. 1861.—*Aspidium heterodon* Schrader, Gött. gel. Anz. 1824:869. 1824.—*Polystichum heterodon* (Schrader) Presl, Epim. 58. 1849.—"*Cyrtomium*" *heterodon* (Schrader) Moore ex C. Chr., Ind. Fil. 197. 1905 [an error by Christensen for *Cyclodium heterodon*].—TYPE: Brazil [Est. Bahia?], exact locality not stated in original publication, Prince *Neowied* s.n. (BR!). There are four sheets that represent possible type material in Brussels; all are identified as *Aspidium heterodon*. I choose the only one with locality data ("in sylvis ad Almadam et in via Felisbertia Prov. Bah." as lectotype; I have been unable to locate this locality on maps. The lectotype shows the distal portion of a frond with six pairs of fertile pinnae, below which are four pairs of sterile pinnae. Another sheet of the type material consists of the stipe and lower portion of the lamina, nearly entirely sterile; this sheet bears Schrader's original description. A third sheet is nearly devoid of data, while a fourth comprises two detached pinnae.

Distribution.—Known only from coastal Brazil, from the states of Bahia, Espírito Santo, and Rio de Janeiro, presumably at low elevations, although no such data are noted on labels.

Discussion.—Only a few collections of var. *heterodon* exist in herbaria. Christensen (1905) placed *Aspidium heterodon* as a doubtful synonym of *Cyclodium meniscioides*. Morton (1939) mentioned the name, but apparently saw no specimens and left it unplaced. It is a readily distinguishable taxon not easily confused with any other in the genus. It is close only to var. *abbreviatum*, which differs by having a single pair of united veins below the sinus and more lobate or incised pinnae. Additional collections of var. *heterodon* are needed to establish its variability.

Venation in *C. heterodon* is intermediate and perhaps evolutionarily transitional between the meniscioid venation found in *C. meniscioides* and free venation found in *C. inerme* and *C. guianense*. To separate these species generi-

FIG. 11. *Cyclodium heterodon* and *C. inerme*. A–C. *C. heterodon* var. *heterodon*, *Brade 18130*, GH, NY. A. Blade apex, fertile frond. B. Largest fertile pinna. C. Largest sterile pinna. D–F. *C. heterodon* var. *abbreviatum*, *Mori* et al. *11351a*, NY. D. Blade apex. E. Rhizome apex. F. Largest pinna. G–I. *C. inerme*, *Jenman* s.n., NY, Guyana, Pacatout, Potaro River. G. Blade apex. H. Pinna. I. Sori and indusia.

cally on the basis of venation, as many have done, is highly artificial and not reflective of their very close relationship.

Additional collections.—BRAZIL. **Espirito Santo**: Alto Limoeiro, *Brade & Appaini 18130* (G, GH, MO, NY). **Rio de Janeiro**: Corcovado, *Gardner 5671* (K); Dois Irmãos, *Cuarto 154* (G, NY—2 sheets); *Glaziou 5392* (B). **State unknown**: Norte de Mina, *Gouverneur s.n.* (US).

4b. **Cyclodium heterodon** (Schrader) Moore var. **abbreviatum** (Presl) A. R. Smith, comb. et stat. nov. (Figs. 11D, F).—*Aspidium abbreviatum* Schrader, non

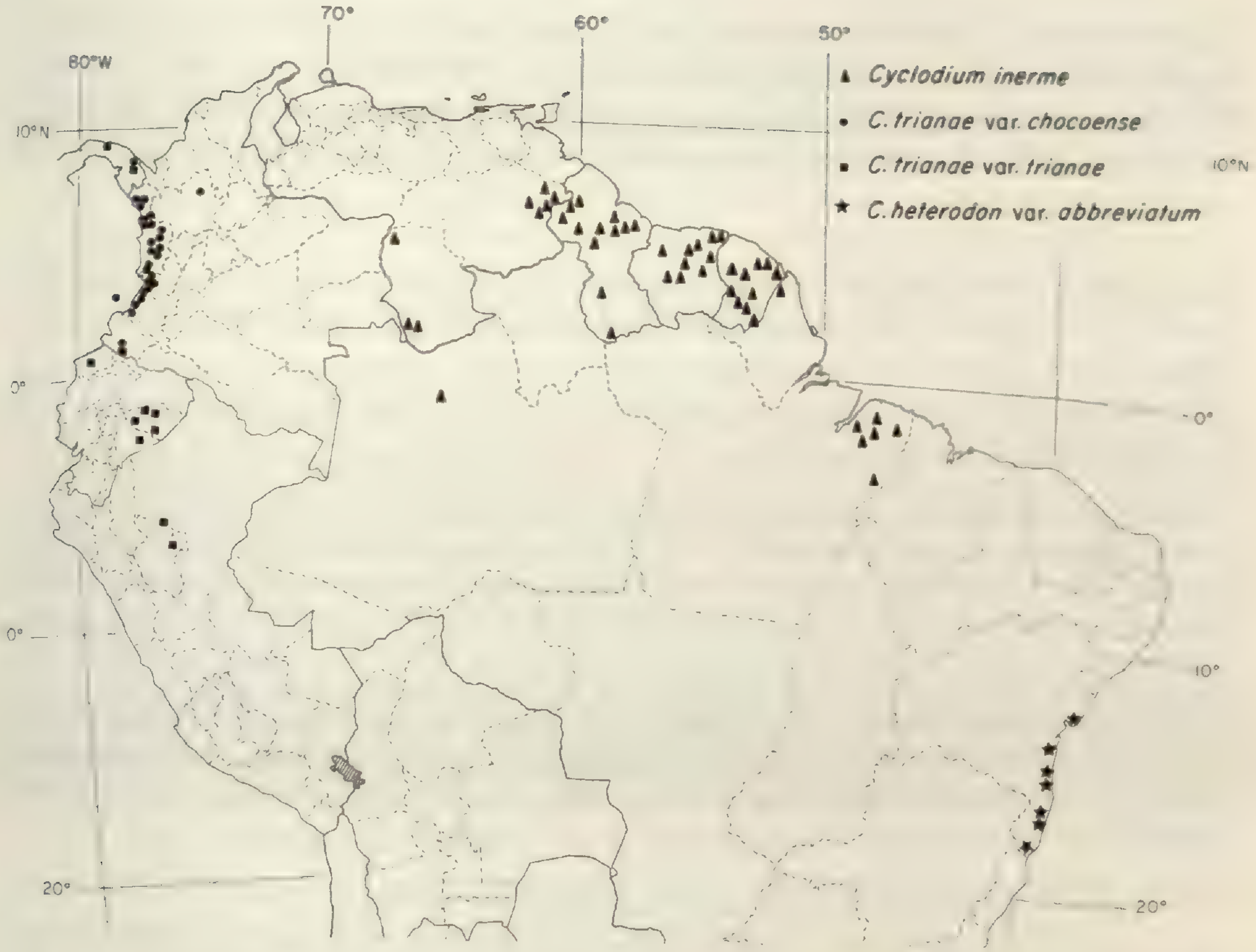


FIG. 12. Distributions of *Cyclodium heterodon* var. *abbreviatum*, *C. inerme*, and *C. trianae*.

Poiret (1816), Gött. gel. Anz. 1824:869. 1824.—*Polystichum abbreviatum* J. Smith, London J. Bot. 1:199. 1842, non Lam. & DC. (1805). (To be considered a nom. nov., but illegit. because it is a later homonym).—*Cyclodium abbreviatum* Presl, Epim. 260. 1849 (to be considered a nom. nov. for *Aspidium abbreviatum* Schrader).—*Nephrodium abbreviatum* (Presl) Fée, Gen. Fil. 306. 1852.—*Cyrtomium abbreviatum* (Presl) J. Smith, Ferns Brit. Foreign, ed. 2. 304. 1877.—*Dryopteris abbreviata* (Presl) Kuntze, Rev. Gen. Pl. 2:812. 1891.—TYPE: Brazil, exact locality not stated in original publication, *Neowied s.n.* (BR!). There are six sheets that represent possible type material in Brussels; five of these are identified as *Aspidium abbreviatum*. Judging from the variation in frond morphology, it seems likely that they represent two, possibly three, independent gatherings of this variety. I choose the one bearing the original description as lectotype; it lacks locality data. Two of the other collections bear the locality "ad fluv. Mucuri" (Mucuri River, which is in southern coastal Bahia), while two others bear the locality "ad Villa Nova d'Almeida" (not located, presumably also Est. Bahia).

Distribution.—Known only from coastal Brazil, from the states of Bahia and Ceará (Fig. 12); a Martius collection (P) labelled as from Pará is more likely from Est. Bahia. This variety presumably occurs only at low elevations, in tropical rain forest; no elevational data are included on labels.

Discussion.—Variety *abbreviatum* is highly variable. Some forms approach *C. inerme* and *C. trianae* var. *chocoense*; others apparently merge with *C. heterodon* var. *heterodon*. From the first two, var. *abbreviatum* generally differs in the presence of at least some veins connivent below the sinus; such anastomoses are often more numerous toward the distal portion of a pinna. In addition, *C. inerme* differs in having smaller, inconspicuous rhizome scales, fewer pairs of lateral pinnae, and more impressed sori; in general *C. inerme* is a smaller species than *C. heterodon*. *Cyclodium trianae* matches *C. heterodon* in size, but can be distinguished by the round-reniform indusia, usual presence of more numerous light yellowish or clear, minute, glandular hairs on the costae abaxially, and generally more deeply cut pinnae.

Additional collections: BRAZIL. **Bahia**: *Blanchet 2208* (G-4 sheets; GH), *2251* (G, P), *s.n.* (BM, G, P); Colonia Leopoldina, *Blanchet 2468* (BM, G, NY, UC, US); Ilheos, *Blanchet 2468* (G), *s.n.* (G); Munic. Una, Maruim, 33 km SW of Olivença on road from Olivença to Buerarema, *Boom et al. 762* (NY); Porto Seguro, *Duarte 6756* (F, MO); *Forte s.n.* (BM); Pau Brasil Biological Reserve, 17 km W from Porto Seguro on road to Eunapolis, *Harley et al. 17173* (M, MO, NY, U, US); *Luschnath 119* (B); between S. Jorge dos Ilheos and S. Pedro d'Alcantara, *Martius* [Herb. Fl. Brasil 325] (BM, G, M, NY, P); S. Jorge dos Ilheos, *Martius* [Herb. Fl. Brasil. 326] (BR, G, M, NY, P); Munic. Maraú, BR 030, Ubaitaba to Maraú, Km 33, *Mori* et al. *11351a* (NY). **Ceará**: *Gardner 1218* (P). **State unknown**: *Riedel s.n.* (BR, GH, both mixed with *C. meniscioides*), *s.n.* (BR, C, FI, M, P, US), "*43*" (GH), "*47*" (GH, NY, mixed with *C. meniscioides* at GH); *Gaudichaud s.n.* (BM); *Duparquier s.n.* (BM).

5. **Cyclodium inerme** (Fée) A. R. Smith, comb. nov. (Fig. 11, G–I).—*Polystichum inerme* Fée, Gen. Fil. 281. 1852.—TYPE: French Guiana, *Leprieur 188* (Herb. Mougeot, not located; isotype P!).

Polypodium subobliquatum Hook., Sp. Fil. 4:240. 1862.—*Nephrodium subobliquatum* (Hook.) Baker, Syn. Fil. 261. 1867—*Dryopteris subobliquata* (Hook.) Kuntze, Rev. Gen. Pl. 2:813. 1891.—*Thelypteris subobliquata* (Hook.) Ching, Bull. Fan Mem. Inst. Biol., Bot. 10:254. 1941.—LECTOTYPE (chosen by Christensen, 1913, p. 81): Surinam, *Hostmann 15* (K!, isolectotype BM!). The other syntype is from Brazil, Pará, *Spruce 36* (K!).

Plants terrestrial; rhizomes short- to moderately long-creeping (up to ca. 7 fronds per 3 cm), 4–8(10) mm diam., at apex with concolorous, castaneous or dark brown, lanceolate scales 3–5(7) mm long, up to 0.8 mm wide, these entire or nearly so, occasionally sparingly denticulate, ascending; fronds (30)40–110(170) cm long, monomorphous or subdimorphous; stipes equalling or somewhat exceeding lamina length, tan to brown, 1–6 mm diam., with a few scattered scales near the base, these similar to those of rhizome but somewhat longer; blade 15–50(75) cm long, 8–25 cm wide, with 9–15(24) pairs of lateral pinnae, these gradually reduced distally to a confluent shallowly pinnatifid apex; pinnae ovate- or elliptic-lanceolate, 4–15(20) cm long, 1.2–3.0(4.0) cm wide, 1.5–3.5 cm distant, incised ¼–½(⅔) the distance to costa, anterior basal segment incised ½–¾ (occasionally cut completely to costa) and sometimes overlapping rachis, often twice as long as posterior basal segment; sterile pinnae often merely broadly crenate and with slightly wider lobes than on fertile pinnae; segments rounded or often blunt at apex, with costules 4–7 mm distant; veins free, arising from costules, the anterior basal one of each segment ending in lamina below sinus,

or running to sinus, the others ending at or above the sinus, 3–6(8) pairs per segment; costae abaxially with a few scattered, appressed, reddish-brown, hair-like scales to 1.0 mm long, these mostly uniseriate, occasionally pluriseriate (2 or 3 cells wide at base); sori up to 6 pairs per segment, discrete, impressed, with a large peltate indusium up to 1.5 mm diam., this tan, glabrous on margin, entire or slightly erose.

Distribution.—Guyana, Surinam, French Guiana, eastern Venezuela (Amazonas, Bolívar), and northeastern Brazil (Pará, Amazonas, Amapa) (Fig. 12). At low elevations, 0–700 m, in forests and along rivers and streams. A collection cited by Christensen (1913) from Rio de Janeiro (*Glaziou 12373*, B, C, G) is very likely from Guyana, possibly collected by Appun and distributed by Glaziou. I refer *Lehmann 3802* (also cited by Christensen), from Cauca, Colombia, to *C. trianae* var. *chocoense*.

Discussion.—This species is most closely related to *C. heterodon* var. *abbreviatum*, from coastal Brazil, and *C. trianae* var. *chocoense*, from the northwestern coast of South America. It is allopatric with respect to both. Another slightly less closely related species is *C. guianense*, with which it overlaps in distribution. *Cyclodium inerme* is most easily distinguished from that species by the lack of true scales along the costae abaxially, by the generally less numerous, wider pinnae, and by having the lowest 1 or 2 veins running to the sinus or ending in the lamina below the sinus (3 or 4 veins ending in lamina below the sinus in *C. guianense*).

Previously, the epithet *guianense* has often been misapplied to this species (e.g., by Kramer, 1978), but the type of *Aspidium guianense* is conspecific with, and prior to, *Nephrodium sancti-gabrieli* Hook.

Two variants are worthy of mention in this rather variable species. Plants from the Kaieteur Plateau (Potaro River Gorge) and Upper Mazaruni River Basin in Guyana have smaller, narrower fronds with more numerous pinnae (e.g., *Cowan & Soderstrom 2157*, US; *Maguire & Fanshawe 23525*, NY, US; *im Thurn s.n.*, K; *Jenman s.n.*, K; *Sandwith 1473*, K; *Tillet et al. 44942*, NY). The largest specimens of the species are from the vicinity of Belém, Brazil (e.g., *Killip & Smith 30335*).

The sole collection seen from the state of Amazonas, Brazil (*Poole 1822*) is very atypical in its deeply pinnatifid pinnae, and thus resembles some specimens of *C. trianae* var. *chocoense*; however it has peltate indusia and costae abaxially that lack the erect, hyaline hairs of that variety.

Representative specimens: GUYANA (32 coll. seen). Takutu Creek to Puruni River, Mazaruni River, *Fanshawe 4902* (BM, K); Mt. Arisaru, *Guppy 6135* (BM); Demerara River, *Jenman 4213* (B, BM, K). SURINAM (31). 9 km N of Lucie River, 12 km W of Oost River, *Irwin et al. 54523* (MO, NY); Ricanau mine near Moengo, Distr. Marowijne, *Kramer & Hekking 3120* (GH, U, VEN); between Km 9.2 and 9.5, Nassau, *Lanjouw & Lindeman 2720* (MO, U). FRENCH GUIANA (15). Rivière Sinnamary à Petit Saut, *Cremers 5389* (Z); Saül, Layon du Grand "Circuit Boeuf Mort," *de Granville 886* (Z); Monts Atachi Bacca, *de Granville 777* (U). BRAZIL (9). **Amapa**: Rio Oiapoque, Igarapé Pontanarrí, *Froes 26025* (UC). **Amazonas** (1): Slopes and summit of Serra de Jacumim, NW of Santa Isabel, *Poole 1822* (NY). **Pará** (7). Tapaná, near Pará, *Killip & Smith 30335* (NY, US); Dto. Acará, Thomé Assú Agua Branca, *Mexia 6042* (BM, GH, K, MO, NY, U, UC, US, Z); Tanaii [Tauaú], ad Rio Acará, *Spruce s.n.* (BM, NY), *36* (K). VENEZUELA (12). **Bolivar** (8): El Dorado, Km 109 SW, *Bernardi 7245* (GH); ridge

leading to Cerro El Picacho, *Steyermark 88601* (NY). **Terr. Fed. Amazonas** (4). Depto. Atures, along Río Cataniapo, 44–45 km SE of Puerto Ayacucho, *Steyermark et al. 122169* (UC); Rios Pacimoni—Yatua, Casiquiare, Piedra Arauicaua, *Maguire et al. 37457* (NY, US).

6. *Cyclodium meniscioides* var. *meniscioides*

Plants terrestrial or low-climbing hemiepiphytes; rhizomes short- to long-creeping (with as few as 1 frond per 3 cm), eventually suberect or erect, 0.5–1.5 cm diam., at apex with brown, linear-lanceolate scales ca. 1–2 cm long, to 0.5–1.5 mm wide, scales subentire to usually denticulate or fimbriate; fronds (35)60–200 cm long, subdimorphous to strongly dimorphous, if the latter then the fertile more erect with shorter, narrower pinnae than the sterile; stipes (1.5)3–10 mm diam., with persistent, usually spreading, linear to linear-lanceolate, denticulate to strongly erose-denticulate, brown scales up to 1.5 cm long; stipes of fertile fronds up to 2 times longer than fertile laminae, those of sterile fronds ± equalling laminae; sterile laminae rarely simple, more often 1-pinnate, with 2–6(9) pairs of lateral pinnae and a ± conform terminal segment; pinnae ovate, 10–25 cm long, (2)4–8 cm wide, lowermost short-stalked to 5 mm, rounded at the base (the basiscopic side often slightly narrower and more cuneate), entire, sinuate, crenate, or deeply serrate; main lateral veins 6–10 mm distant, each with 4–7 pairs of arcuately ascending veinlets arising from costules, anastomosing and giving rise to an excurrent veinlet; costae, veins, and often lamina abaxially with appressed, brownish, septate, hairlike scales to 1 mm long, these uniseriate or only a few cells wide at base; fertile laminae rarely simple, usually pinnate, with 2–10(12) pairs of lateral pinnae and a conform terminal segment; pinnae linear-oblong to less frequently ovate, 4–11(22) cm long, 1.0–3.5(6.0) cm wide, sinuate, crenate, or shallowly lobed less than ⅕ the distance to costa; venation similar to that of sterile fronds but veins more crowded, with main lateral veins 3–5 mm distant; sori round, discrete or often crowded, medial on veins, at maturity often confluent, in 4–9 rows between costa and margin, biseriate between the main veins, with very large peltate indusia 1.0–2.0 mm diam., these tan, usually ciliate along margin and sometimes also on surface, cilia up to 0.4 mm long.

6a. *Cyclodium meniscioides* var. *meniscioides* (Fig. 7, E–K)

Cyclodium meniscioides (Willd.) Presl, Tent. Pterid. 85. 1836.—*Aspidium meniscioides* Willd., Sp. Pl. 5:218. 1810.—*Nephrodium meniscioides* (Willd.) J. Smith, Hook. J. Bot. 4:188. 1841.—*Dryopteris meniscioides* (Willd.) Kuntze, Rev. Gen. Pl. 2:813. 1891, as "menisciodes" (non *D. meniscioides* (Liebm.) C. Chr., 1905.—*Stigmatopteris meniscioides* (Willd.) Kramer in Kramer & van Donselaar, Koninkl. Nederl. Akad. Wetensch. Proc., C, 71:521. 1968.—TYPE: Brazil, *Hoffmannsegg* s.n. (B-Hb. Willd. 19737, microfiche at UC!).

Aspidium confertum Kaulf., Enum. Fil. 232. 1824.—*Cyclodium confertum* (Kaulf.) Presl, Tent. Pterid. 85. 1836.—*Nephrodium confertum* (Kaulf.) J. Smith, Hook. J. Bot. 4:188. 1841.—"*Cyrtomium*" *confertum* Presl ex C. Chr., Ind. Fil. 197. 1905 [an error by Christensen for *Cyclodium confertum*].—*Dryopteris meniscioides* (Willd.) Kuntze var. *conferta* (Kaulf.) Morton,

Bull. Torrey Bot. Club 66:51. 1939.—TYPE: French Guiana ["Cayenna"], *Richard s.n.* (LE?).

Soromanes integrifolium Fée, Hist. Acrost. (Mém. Fam. Foug. 2) 82, pl. 42. 1845.—TYPE: Brazil, specimen in herbarium of A. Braun (B?). Illustration of sterile frond is of this species; fertile frond is *Polybotrya serratifolia* (Fée) Klotzsch (R. Moran, in litt.).

Aspidium hookeri Klotzsch, Linnaea 20:364. 1847.—TYPE: Based on Hooker & Greville, Icon. Fil. pl. 121, 1829, an illustration of *Aspidium confertum* Kaulf., supposed by Klotzsch to be different from the original species of Kaulfuss.

Campium molle Copel., Philipp. J. Sci. 37:390, figure 41, pl. 29. 1928.—*Bolbitis mollis* (Copel.) Ching in C. Chr., Ind. Fil. Suppl. 3:49. 1934.—TYPE: Brazil [said to be "Ceylon"], *Gardner s.n.* (K!). See Hennipman (1977) for discussion.

Distribution.—Trinidad, French Guiana, Surinam, Guyana, Venezuela (Amazonas, Apure, Bolívar, Delta Amacuro, Táchira), Colombia (Amazonas, Putumayo, Vaupés), Ecuador (Pastaza, Santiago-Zamora), Peru (Amazonas, Junín, Loreto, San Martín), Bolivia (La Paz), nearly throughout Brazil, northeastern Argentina (Corrientes), and Paraguay (Fig. 8). A collection labelled as from Dominica (*Othmar s.n.*, M) is erroneously labelled and is really from Trinidad, where Othmar also collected. *Cyclodium meniscioides* occurs at low elevations, 0–700(1150) m, in gallery forests, along streams, in waterlogged soils and swamps. In Guyana, it was described by Jenman (1909, p. 203) as "abundant everywhere . . . throughout lower forest regions."

Discussion.—This is the most widely distributed and variable of all cyclodiums. Morton (1939) distinguished var. *confertum* by its greater dimorphism. In fact, there is a continuum from plants showing very little dimorphism between fertile and sterile fronds to plants that have strongly dimorphic fronds. These extremes are to be found intermingled, nearly throughout the range of the species, and I am convinced that dimorphism by itself is not a useful or meaningful character for constructing a taxonomy in this species.

By far the most typical condition is for fronds to be either entirely sterile or fertile. However many collections have been made that show various combinations of fertile and sterile pinnae on the same frond, or even sterile and fertile regions on the same pinna (see Jenman, 1909). Two collections (Essequibo River, *Jenman s.n.*, NY; Bolivia, *Williams 1202*, NY) show portions of fronds with broad sterile pinnae along one side of the rachis and narrow fertile pinnae on the opposite side. A specimen from Bahia, Brazil (*Luschnath s.n.*, BR), shows a frond with the proximal two pairs of pinnae sterile and the distal pinnae fertile. Other collections have individual pinnae sterile in the basal region and highly contracted and fertile at the tip (Brazil, Mato Grosso, *Santos* et al. *R1486*, US); Guyana, *Appun 166*, K; Guyana, *Maguire & Fanshawe 22821*, NY). Still another variant has individual pinnae with the basal region fertile and the distal region dilated and sterile (Paraguay, *Fiebrig 4984*, K; Brazil, Bahia, *Blanchet 2519*, BM). These variations occur throughout the range of the species and indicate the ease

with which plants assume a different developmental mode. The dimorphism must be genetically based, but it is perhaps under the control of relatively few genes; the various manifestations discussed indicate that its expression is possibly under environmental or nutritional control.

Within var. *meniscioides*, I have tried without success to find suites of characters that would circumscribe some of the more distinctive elements. However, there exist some trends over the range of the variety that are worthy of note:

1) Most plants from Guyana have relatively few pairs of lateral ± entire sterile pinnae and are strongly dimorphic. The most extreme specimen is *Jenman H.29* (K), which has simple sterile and fertile fronds. Many other collections have only two or three pairs of lateral pinnae. Only six of the 57 Guyanan collections seen have weakly dimorphic fronds with sori not confluent at maturity: *de la Cruz 1177*, NY, US; *1921*, MO, NY, US; *3119*, GH, NY, US; *3847*, NY; *Jenman s.n.*, NY, US, Demerara River; and *Jenman* s.n., NY, Mt. Raywa, Isorooroo River. It is of interest that other collections of *de la Cruz 1921* (F, GH) represent the strongly dimorphic form with contracted fertile pinnae. Also, *de la Cruz 3844* (GH, MO, NY, US), collected at the same time and place as *3847*, is strongly dimorphous. The collection of both morphotypes at the same locality and the complete lack of any additional correlating characters offer further evidence of the taxonomic weakness of this character.

The same phenomenon occurs in other regions, for example Colombia, where both strongly dimorphic (*Soejarto & Cardozo 740*, GH) and very weakly dimorphic (*Soejarto & Cardozo 718*, GH) plants were collected at the same time and place. In the vicinity of Pará, Brazil, *Killip & Smith 30316* and *30331* were collected together and are, respectively, strongly dimorphic and weakly dimorphic plants.

2) Nearly all collections from central and southern Brazil (D.F., Goiás, Mato Grosso, Minas Gerais, Piauí, and São Paulo), Paraguay, and Argentina have sterile pinnae that are more deeply and sharply serrate than specimens from the rest of the range. All of the specimens from this region are moderately to strongly dimorphic, but the sori are not usually completely confluent at maturity; sterile pinnae are usually crenately or serrately incised (as opposed to entire or subentire in many Guyanan collections). None of the specimens has been recorded as climbing. Elsewhere in Brazil, in Est. Pará and coastal Bahia, most specimens have entire to subentire sterile pinnae. Specimens from the Amazon basin of Brazil tend to be variable and intermediate between those from Guyana and those in category (2) in the margins of sterile pinnae.

3) Many specimens, from Amazonian Brazil, Colombia, and eastern Peru, have been recorded as low hemiepiphytes. For example, *Plowman 2452* is described as climbing two meters into trees; a sheet of *Conant 1008* has an erect, climbing caudex 40 cm long that bears tightly packed bases of old fronds and roots throughout. Furthermore, about half of the collections from this region have sterile pinnae that are only slightly dimorphic, with discrete sori at maturity. However, both strongly dimorphic and terrestrial plants do occur in this same region. Some of these specimens also have noticeably lighter-colored (light brown to tan), longer, and stiffer scales at the rhizome apex, e.g., *Plowman 2452* and *Soejarto & Cardozo 718* from Colombia.

Representative specimens: TRINIDAD (6 coll. seen). Aripo Road via Arima, *Broadway 5758* (MO); Arena Government Forests, *Broadway 6009* (MO); *Othmer 117* (M). FRENCH GUIANA (14). Fleuve Ouaqui (Saut Macaque), *de Granville 1761* (NY, U, Z); Saut Mais, 20 km E of Saül, *de Granville 3078* (Z); Baduel, *Leprieur 27* (GH, US), *131* (G). SURINAM (25). 2 mi from Mapane Forest Camp 8, *Bierhorst S70* (GH); Emmaketen, *Daniëls & Jonker 1259* (U); Nassau Mts., creek near Km 10.5, *Lanjouw & Lindeman 2849* (U, US). GUYANA (57). Pomeroon Dist., Kamwatta, *de la Cruz 1174* (GH, NY, US), *1177* (NY, US) vicinity of Bartica, on Essequibo River, *de la Cruz 1921* (F, GH, MO, NY, US); Amakura [Amacuro] River, Northwest Distr., *de la Cruz 3488* (F, GH, MO, NY, UC, US). VENEZUELA. **Apure** (1): Dto. Pedro Camejo, along Río Meta at Fundo El Algarrobo, *Davidse & González 14294* (MO, VEN). **Bolívar** (8): slopes of Cerro Pijiguao, *Wurdack & Monachino 41294* (F, GH, NY, US, VEN). **Táchira** (1): 10 km E of La Fundación, around Repressa Dorada, *Liesner & González 10383* (MO, UC). **Terr. Fed. Amazonas** (2): 3 km E of San Carlos de Río Negro, *Liesner 3535* (MO, UC). **Terr. Fed. Delta Amacuro** (2): Lower Orinoco, Eleanor Creek, *Rusby & Squires 377* (NY, US), *379* (BM, F, GH, NY, US). COLOMBIA. **Amazonas** (6): 7 km N of Leticia, *Plowman 2452* (BM, F, GH, NY). **Putumayo** (1): Río Putumayo en Puerto Ospina, *Cuatrecasas 10563* (US). **Vaupés** (2): Yurupari, Río Vaupés, ±350 km above Mitú, *Cuatrecasas 6996* (F, US). ECUADOR. **Napo** (1): Camino Puyo-Tena, ca. 10 km S of Río Negro, *Moran 3577* (UC). **Pastaza** (3): Río Bobonaza, between Cachitama and outlet of Río Bufeo, *Øllgaard 34742* (AAU). **Santiago-Zamora** (1): Macuma, on Macuma River, S of Chiquaza, *van der Werff 604* (U). PERU. **Amazonas** (1): near Pongo Mori, Río Comain, on trail to Kusu, *Berlin 949* (MO, UC). **Junín** (2): Pichis Trail, Santa Rosa, *Killip & Smith 26165* (GH, NY, US). **Loreto** (15): 7 km SW of Iquitos, *Croat 18579* (MO). **San Martín** (2): Palo Blanco, Mariscal Caceres, Tocache Nuevo, *Schunke 5610* (U). BOLIVIA. **Beni** (1): ca. 18 km E of Riberalta, *Solomon 6186* (UC). **La Paz** (2): San José, *Williams 1191* (NY, GH, US), *1202* (NY). BRAZIL. **Acre**: reported by Windisch, Bradea 3(5):29–30. 1979. **Amazonas** (10): Reserva Ducke, Km 26, Manaus-Itacoatiara Road, *Conant 914* (GH, NY). **Terr. Amapa** (3): Rio Oiapoque, 4 km SE of Clevelandia, *Irwin & Westra 47168* (NY, U). **Bahia** (5): Ilheos, *Blanchet 2519* (BM, G, NY). **Ceará** (1): Serra de Maranguape, *Ule 9128* (G, UC). **D.F.** (3): ca. 15 km E of Brasilia, *Irwin & Soderstrom 5739* (F, MO, NY, US). **Goiás** (4): Goiania, *Brade 15360* (NY). **Mato Grosso** (13): 1 km E of Km 256, Xavantina-Cachimbo Road, *Philcox et al. 3710* (AAU, K, MO, UC, US). **Minas Gerais** (1): 1844, *Widgren s.n.* (M). **Pará** (14): Ilha de Colares, Munic. da Vigia, *Black 54-16942* (BM). **Piauí** (1): *Gardner 2759* (K). **São Paulo** (5): Munic. Mogi-Guaçú, na Fazenda Compininha, *Gottsberger 34-11067* (Z). ARGENTINA. **Corrientes** (2): Depto. Ituzaingó, Estancia "El Plata," *Meyer 6219* (A), *6280* (A, U, UC), *6281* (U). PARAGUAY. **Amambay** (3): Sierra de Amambay, Estrella, *Hassler 10131* (G, MICH), *10131a* (G, K, MICH). **Caaguazú** (2): vicinity of Caaguazú, *Hassler 9049* (BM, G, GH, MICH, NY, UC); Canendiyu (2): Igatimí, *Hassler 4783* (BM, G, NY, UC). **Guaira**?: Santa Barbara, near Villa-Rica, *Balansa 325* (G). **Alto Parana**: in regione fluminis Alto Paraná, *Fiebrig 6344* (BM, G, GH, K, US).

6b. **Cyclodium meniscioides** (Willd.) Presl var. **paludosum** (Morton) A. R. Smith, comb. et stat. nov.—*Dryopteris paludosa* Morton, Bull. Torrey Bot. Club 66:50. 1939.—*Stigmatopteris paludosa* (Morton) R. & A. Tryon, Rhodora 83:136. 1981.—TYPE: Colombia, Dept. Antioquia, Puerto Berrio, 130 m, *Pennell 3723* (NY! fragment US!).

Differs from var. *meniscioides* in its smaller fronds (sterile pinnae to 11 cm long, 3.0 cm broad) with more numerous lateral pinnae (ca. 12 pairs); pinna margins sharply and regularly serrulate; and veins more closely placed on the sterile pinnae (10–11 pairs per 3 cm).

Distribution.—Known only from the type and a single collection from Peru; low elevations, ca. 130–160 m. The type was recorded as growing in a swamp.

Discussion.—The paucity of collections makes it difficult to assess the taxonomic distinctness of this taxon, but it seems to represent no more than a minor variant within the widespread and variable *C. meniscioides*. It differs from var. *meniscioides* only in a few quantitative characteristics that tend to be highly plastic in this species. Morton (1939) distinguished *Dryopteris paludosa* by its

strongly ascending, smaller pinnae and serrulate pinna margins, but these conditions also occur in var. *meniscioides*; however in that variety, the pinna margins, if serrate, are generally more coarsely serrate, a reflection of the main lateral veins being more widely spaced.

The Peruvian specimen cited is a nearly perfect match for the Colombian type.

Additional collection: PERU. **Loreto**: Bersalles-Iquitos, *Vargas 11476* (GH).

6c. **Cyclodium meniscioides** (Willd.) Presl var. **rigidissimum** (C. Chr.) A. R. Smith, comb. et stat. nov.—*Cyclodium rigidissimum* C. Chr., Bot. Tidsskr. 25: 79. 1902.—*Aspidium rigidissimum* C. Chr., Bot. Tidsskr, 25:79. 1902, nom. inval. (cited in synonymy, as an alternative name).—TYPE: "Brazil, *Glaziou 12374*" [according to Christensen, 1902, but actually collected in Guyana by *Appun* and distributed by Glaziou under his number 12374, as shown by an isotype in Geneva; *Appun 1176*, K, may be part of the same gathering] (holotype C-3 sheets! isotypes G-3 sheets!).

Differing from var. *meniscioides* in the characters given by Christensen: its very rigid texture, more numerous pinnae (15–16 pairs) that are approximate and imbricate in sterile fronds, and by the very distinct and raised veins. To this, it may be added that the distal pinnae of both fertile and sterile fronds are more or less gradually reduced with the lamina terminating in a more triangular apical pinna that may be lobed at the base.

Distribution.—Known only from Guyana, in rain forests at lower elevation (ca. 500 m, K.E.R. 167, the only collection with ecological data). A specimen from Venezuela, Edo. Bolívar (*Aymard* et al. *4196*, UC) approaches this variety.

Discussion.—Christensen himself was unsure of the status of his species, referring to it in his discussion as a "subspecies of *C. meniscioides*." It has the venation, costal scales, ciliate indusia (except the type, which seems to have eciliate indusia), and erose-denticulate rhizome scales of var. *meniscioides*. It also seems related to, perhaps even evolutionarily transitional toward, *C. akawaiorum*, which see for differences.

The only information about the ecology of this variety derives from K.E.R. 167, which was said to be a "common climbing fern, or rhizome creeping initially to a support, then climbing. Young plants with a ± erect stock."

Additional collections: GUYANA. *Appun 1176* (K); E side of Waruma river, ca. 7.5 mi NNW of N prow of Mt. Roraima, vicinity of Camp 4, on Waruma Trial, 5°21′20″N, 60°45′50″W, *Warrington* et al. *K.E.R. 167* (K-3 sheets; UC).

7. **Cyclodium rheophilum** A. R. Smith, sp. nov. (Figs. 9, A, B; 10).—TYPE: French Guiana, Upper Oyapock, Mt. St. Marcel, S slope, on exposed, flooded, moss-covered rocks, 260 m, 29 July 1975, *de Granville 2586* (holotype F!; isotype Z!).

Species ex affinitate *C. inerme* et *C. guianense*, ab utroque pinnis perangustis, margine tantum crenato, venulis paucioribus, et habitu rheophytico distincta; a *C. inerme* pinnis lateralibus numerosioribus et a *C. guianense* costis epaleatis differt.

Plants rheophytic; rhizomes short-creeping (ca. 12 fronds per 3 cm), 5 mm diam., at apex with brownish, dull, ovate-lanceolate, slightly erose, appressed scales 3–4 mm long, 1 mm wide; fronds 30–45 cm long, monomorphous; stipes ca. ¼–½ the lamina length, stramineous to tan, 1.5–2.5 mm diam., with a few scattered scales near the base, these to 3 mm long, ovate, slightly erose and with a somewhat darker center; lamina to 30 cm long, 15 cm wide, with up to 20 pairs of lateral pinnae, these gradually shortened distally to a confluent pinatifid apex; pinnae linear-lanceolate, up to 9 cm long, 0.5–0.7 cm wide, 1.0–1.5 cm distant, inequilateral at the base, the upper side truncate and often slightly auriculate, narrowly cuneate at the base, the lowermost pinnae stalked 1–2 mm, pinna margins cartilaginous and crenate, apex acuminate and serrate; primary veins (arising from costae) 1-forked near the base (sterile pinnae) or with 1 or 2 acroscopic veinlets and 0 or 1 basiscopic veinlet, all veins free; costae glabrous abaxially or with a few scattered, minute, appressed, reddish-brown, septate, hairlike scales to 0.3 mm long; sori confined to first acroscopic veinlet or sometimes also on basiscopic veinlet, discrete, impressed, with a large, thick, peltate indusium up to 1 mm diam., this with a tan center and darker, minutely glandular margin.

Discussion.—This species is known only from the type collection. Its closest relationship is with *C. inerme* and *C. guianense*. From both of these it differs in its very narrow crenate pinnae and by the reduced number of veinlets from the main lateral veins. From *C. guianense* it differs in lacking costal scales, and from *C. inerme* it differs in the much more numerous lateral pinnae. Because of the paucity of collections, it is difficult to assess the significance of some of the other differences among these three species, but only the indusium of *C. rheophilum* has a dark margin that is fringed with minute glands.

With its narrow pinnae and small fronds, this new species is obviously adapted to a streamside or rheophytic existence. It is not known whether the species will tolerate long periods of inundation or is only able to withstand sporadic flooding. No other member of the genus has been recorded as occupying this habitat.

4497

8. **Cyclodium seemannii** (Hook.) A. R. Smith, comb. nov. (Fig. 13, A–C).—*Aspidium seemannii* Hook., Sp. Fil. 4:34, pl. 230. 1862.—*Polystichum seemannii* (Hook.) J. Smith, Hist. Fil. 220. 1875 (as "*semani*").—*Dryopteris seemannii* (Hook.) Kuntze, Rev. Gen. Pl. 2:813. 1891, non C. Chr. (1905).—TYPE: Colombia ["Panama"], Dept. Chocó, Bay of Ardita, *Seemann* s.n. (K!; probable isotypes P!-2 sheets).

1885 - 24935 14936

Rhizome not known; complete fronds not seen, probably ca. 1 m long, monomorphous; stipes not known; complete lamina not seen, bipinnate-pinnatifid, probably ca. 50 cm long or more, 30 cm wide, anadromous throughout, with ca. 15 pairs of lateral pinnae, these gradually shortened distally to a deeply pinnatifid apex; rachises brownish, stout, to ca. 4 mm broad, with relatively uniform short hairs 0.1–0.3 mm long; pinnae ascending, pinnate–pinnatifid, up to 25 cm long, 8 cm wide; pinnules up to 4.5 cm long and 1.0 cm wide, obtuse at tip, inequilateral, largest ones stalked to 3 mm, deeply pinnatifid or with basal acroscopic segment free and narrowed at its base, excised or cuneate basiscopically, with up to 12 pairs of blunt segments, distally the pinnules becoming

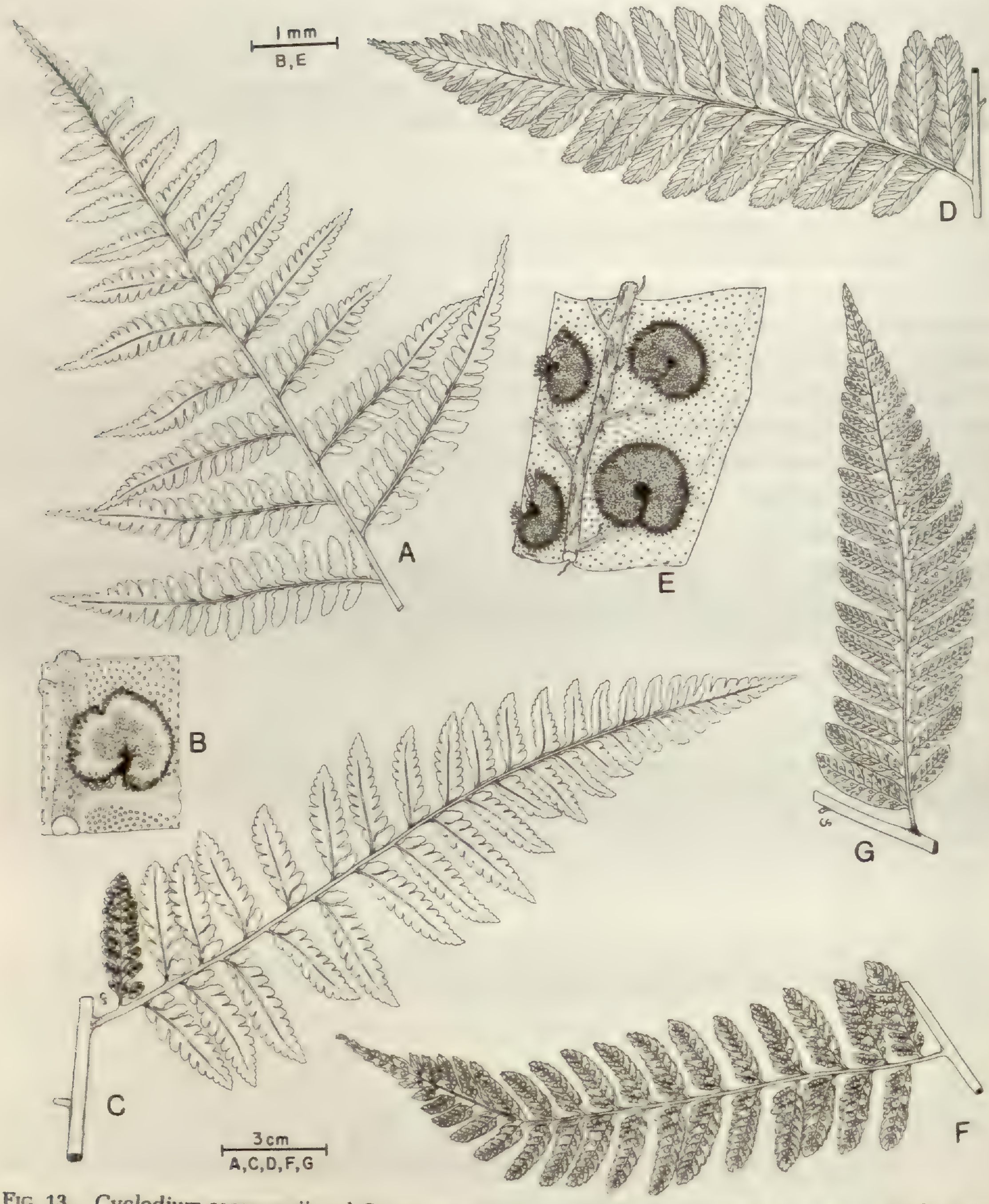


FIG. 13. *Cyclodium seemannii* and *C. trianae*. A–C. *C. seemannii*, *Seemann s.n.*, K. A. Blade apex. B. Sorus and indusium; note sessile lamina glands. C. Largest pinna. D–F. *C. trianae* var. *trianae*, *Mexia 8452*, UC. D. Sterile pinna. E. Sori and indusia. F. Fertile pinna. G. *C. trianae* var. *chocoense*, *Lellinger & Sota 343*, US, larger pinna.

crenate to subentire (but blade still bipinnate in distal third); acroscopic pinnules of proximal pinnae slightly longer than basiscopic pinnules, the basal basiscopic one noticeably shortened to about ⅔ the length of the basal acroscopic segment; veins all free, pinnate in the ultimate segments, 2 or 3 pairs per segment; costae abaxially with rather dense, reddish-brown, minutely septate hairs 0.2 mm long, lacking scales; lamina subcoriaceous, abaxially with numerous sessile, shining,

yellowish to orangish, globose glands; sori discrete, 2–3 pairs in each ultimate segment, not impressed, with a large, caducous, round-reniform, densely glandular indusium up to 1.3 mm diam.

Discussion.—This species is known only from the type collection, probably collected nearly sea level. It is most closely related to *C. trianae*, especially var. *trianae*, differing primarily in having dense, shiny glands on the lamina abaxially. Like *C. trianae*, the indusium in *C. seemannii* is round-reniform, with a distinct sinus. The pubescence of *C. seemannii* is longer and more persistent on the more distal axes than in *C. trianae*, but additional material is needed to assess the value of this character.

Christensen (1920) did not mention this species in his monograph of *Dryopteris*; perhaps he never saw specimens of it.

9. *Cyclodium trianae*

Plants terrestrial; rhizomes creeping, 6–15 mm diam., at apex with concolorous, entire, castaneous, linear-lanceolate scales ca. 4–8 mm long, up to 0.5 (1.0) mm wide; fronds (75)100–150 cm long, monomorphous or only slightly dimorphous (sterile fronds tending to have less deeply incised pinnae with wider segments); stipes stramineous to brownish, equalling the laminae or up to 2.2 times longer than laminae, 3–7 mm diam., with scattered, spreading scales near the base similar to those of rhizome; laminae 35–60 cm long, (12)20–30 cm wide, with 12–18 pairs of lateral pinnae, these gradually reduced distally to a confluent deeply pinnatifid apex; pinnae lanceolate, deltate-lanceolate, or ovate, (8)12–20 cm long, (2)3–6 cm wide, deeply pinnatifid (infrequently incised only ca. ½ the distance to costa), pinnate, or pinnate-pinnatifid, the anterior basal segment (pinnule) often twice as long and more deeply incised than the basiscopic basal segment; segments (pinnules) rounded to acute at tip, up to 1.5 cm wide, entire to crenate or the largest ones pinnatifid nearly to costule; veins free, the basal pair from adjacent segments meeting margin at or just above the sinus, forked at the sorus in larger segments; costae and sometimes costules abaxially with scattered, appressed, reddish-brown, septate, hairlike scales 0.5–2.0 mm long (at most 2 or 3 cells wide at base and with a long, uniseriate tip), also with clear or light yellowish, erect, glandular hairs 0.1–0.2(0.3) mm long; sori discrete, not or faintly impressed, with a round-reniform (rarely truly peltate) indusium up to 1.8 mm diam., this usually brown or reddish-brown, glabrous or often ciliate on margin and glandular-hairy on surface.

Discussion.—*Cyclodium trianae* has never before been placed with its appropriate congeners. Christensen (1920) included it with a group of species now variously placed in *Polystichopsis* (*ochropteroides, lurida, pubescens, chaerophylloides*), *Lastreopsis* (*amplissima*), and *Arachniodes* (*macrostegia, denticulata, rigidissima, leucostegioides, formosa*). He acknowledged the possibility that it might be allied to species of *Stigmatopteris* subg. *Peltochlaena* [= *Cyclodium*] because of its similar lamina shape, venation, shape of lobes, and pinnae narrowed at the base. Morton (1939) overlooked or ignored *C. trianae*.

Along with *C. seemannii*, *C. trianae* can be distinguished from all other members of the genus by the round-reniform (rather than peltate) indusia. These may

sometimes appear "pseudopeltate," with the indusial stalk off-center or lateral; however, most specimens show a distinct, narrow sinus leading to or toward the point of attachment of the stalk. This is often difficult to discern in older fronds where indusia are curled at the margin or lost. Younger fronds generally show the indusial form nicely, as for example in the type of *C. trianae* (also *Mexia 8452*; *Holm-Nielsen* et al. *21985*) and in many specimens of var. *chocoense* (e.g., *Lehmann 4435*; *Killip 11786*; *Killip 35287*, type).

The type of *C. trianae* and *Mexia 8452* are rather densely long-hairy on the costae abaxially, and in this respect are similar to many specimens of var. *chocoense*. Specimens from the Amazonian side of Ecuador and Peru tend to be less pubescent, but are nearly identical to the type in blade dissection.

9a. *Cyclodium trianae* var. *trianae* (Fig. 13, D–F)

Cyclodium trianae (Mett.) A. R. Smith, comb. nov.—*Aspidium trianae* Mett., Ann. Sci. Nat. Bot., V. 2:243. 1864.—*Nephrodium trianae* (Mett.) Baker, Syn. Fil. 286. 1867.—*Dryopteris trianae* (Mett.) Kuntze, Rev. Gen. Pl. 2:814. 1891.—TYPE: Colombia, Prov. de Barbacoas [Nariño], via de Túquerres, *Triana* "32" (B!; isotype BM! photos UC! US!).

Nephrodium firmifolium Baker, Syn. Fil., ed. 2. 501. 1874.—*Dryopteris firmifolia* (Baker) Kuntze, Rev. Gen. Pl. 2:812. 1891.—TYPE: Peru [Dept. San Martín], Mt. Guayapurima, prope Tarapoto, *Spruce 4662* (K! isotypes BM! BR! P).

Stipes ± equalling or only slightly exceeding lamina length; laminae 2-pinnate-pinnatifid at base, apex deeply pinnatifid to pinnatisect; larger pinnae 4–6 cm wide, with pinnules constricted to a narrow stalk at the base, not strongly decurrent; pinnules (at least the largest ones) shallowly to deeply pinnatifid, more deeply so along acroscopic margin, sometimes with a nearly free anterior basal segment; lamina texture chartaceous to subcoriaceous; indusia glabrous on surface, ciliate along margin.

Distribution.—Known from Panama (Darién), Colombia, Ecuador, and Peru (Fig. 12); low elevations (100–700 m) in dense, primary rain forest.

Discussion.—When additional and more complete collections can be made, it may prove difficult to draw even varietal boundaries between var. *trianae* and var. *chocoense*. Nevertheless, var. *trianae*, the more dissected of the two, has been found in Ecuador and Peru (primarily on the Amazonian side), areas where var. *chocoense* is not yet known. Furthermore, var. *chocoense* is common along the Pacific coast of Colombia, while var. *trianae* is known from this region only from the type.

Sterile specimens of *C. trianae*, especially var. *trianae*, look remarkably like certain bipinnate species of *Polybotrya*, and as discussed in the section on relationships, this species may provide the closest extant link to that genus.

Additional collections: PANAMA (1 coll. seen). **Darién** (1): Along headwaters of Río Tuquesa, ca. 2 km from Continental Divide, *Croat 27128* (US). COLOMBIA (1). **Nariño**: only the type. ECUADOR (12). **Esmeraldas** (1): Parroquia de Concepción, Playa Rica, *Mexia 8452* (BM, UC). **Morono-Santiago** (1): Taisha, 8–10 km NNW of military camp, *Brandbyge & Asanza 31930* (AAU). **Pastaza** (10): Near Canelos, *Mexia 6894* (GH, UC, US); Finca El Valle de Muerte on Río Curaray, ca. 10 km E of

Curaray (Jesús Pitishka), *Harling & Andersson 17617* (GB); Curaray, near posto militar, *Harling & Andersson 17534, 17387* (GB); Curaray, NE of Destacamento, *Holm-Nielsen et al. 21985* (AAU), *21987* (AAU), *22029* (AAU), *22061* (AAU); Tzapino, Waorani (Aucas), *Jaramillo & Coello 3648* (AAU); Río Bobonaza, below Montalvo (Limón), *Øllgaard* et al. *34528* (AAU). PERU (2). **Loreto** (1): Balsapuerto, lower Río Huallaga basin, *Killip & Smith 28540* (NY, US).

9887

9b. **Cyclodium trianae** var. **chocoense** A. R. Smith, var. nov. (Fig. 13G).—TYPE: Colombia, Chocó, Corcovada region, upper Río San Juan, ridge along Yeracüí Valley, 200–275 m, *Killip 35287* (US! isotype COL).

Stipes quam lamina usque ad 2.2 plo longiorus; laminae profunde pinnato-pinnatifidae vel bipinnatae; pinnulae integrae vel crenatae, decurrentes; pinnae fertiles (2)3–4(5) cm latae; laminae subcoriaceae vel coriaceae; costae infra plerumque pilis numerosis hyalinis 0.1–0.2 mm longis; indusia ad marginem ciliata.

Distribution.—Known only from Panama (Darién) and the Pacific coast of Colombia (Fig. 12); low elevations (0–200 m, rarely to 1450 m) in dense, primary rain forest. Only five (out of 41) collections are from above 250 m (*Lellinger & de la Sota 211, 253*; *Duke 15522*; *Alverson 140, 143*).

Discussion.—This variety is clearly closely allied to *C. inerme*, and most specimens of var. *chocoense* have been so determined (as *Dryopteris guianensis*, a misapplied synonym) in herbaria. From *C. inerme*, var. *chocoense* differs in the generally larger fronds with wider, more deeply pinnatifid to pinnate pinnae, in the more numerous and longer hyaline hairs on the costae abaxially, in the veins often forked in the ultimate segments, and in the round-reniform indusia (with a narrow sinus) that are often ciliate on the margin and sometimes on the surface. Another close relative is *C. heterodon* var. *abbreviatum*. *Cyclodium trianae* is allopatric with respect to both *C. heterodon* and *C. inerme*.

Additional collections: PANAMA (4 coll. seen). **Darién** (2): Hills of Sperdi, near Puerto Obaldía, *Pittier 4413* (GH, US), *4415* (GH). **Panama** (1): Cerro Jefe, *van der Werff & van Hardeveld 6986* (UC). **San Blas** (1): El Llano–Carti Road, *de Nevers & Herrera 4001* (UC). COLOMBIA (41). **Antioquia** (2): Vicinity of Planta Providencia, 28 km SW of Zaragoza, valley of Río Anorí, *Alverson et al. 140* (MO), *143* (MO, NY). **Cauca** (2): Río Micay, at Guayabal, *Cuatrecasas 14130* (US); Timbiquí, *Lehmann B.T. 433* (US). **Chocó** (19): Río San Juan, hill in front of Palestina, *Cuatrecasas 21421* (F, US); Hydro Camp No. 8, peak over Río Curundu, *Duke 15522* (US); Río Serrano, 4–6 km above Guayabal, *Forero et al. 1342* (COL, NY); Quebrada La Sierpe, *Forero & Jaramillo 4450* (COL, US); Road Andagoya–Condoto, near Andagoya, *Forero et al. 5269* (COL, US); Pan American Hwy, ca. 10 km W of Las Animas, *Gentry & Renteria 24057* (US); near jct. Río Condoto and Río San Juan, *Killip 35084* (COL, US); Bahía Solano, near Ciudad Mutis, *Killip & Garcia 33622* (COL, US); near Punta San Francisco Solano, ca. 10 km NE of Puerto Mutis, *Lellinger & de la Sota 82* (COL, US); NW side of Alto del Buey, *Lellinger & de la Sota 211, 253* (COL, US); 1.0–1.5 km NW of El Valle, *Lellinger & de la Sota 343* (COL, US); Mojarras de Tadó, 8.5 km E of Istmina, *Lellinger & de la Sota 417* (COL, US); Río El Salto, 9 km W of Andagoya, *Lellinger & de la Sota 478* (COL, US); Upper Río Truando, 5 km NE of confluence of Río Nercua, *Lellinger & de la Sota 562* (US); Upper Río Truando, 2 km SSW of confluence of Río Nercua, *Lellinger & de la Sota 590* (US). **Nariño** (2): Quebrada La Toma, on Río Telembi, above Barbacoas, *Ewan 16861* (BM, US); E side of Gorgona Island, *Killip & Garcia 33172* (COL, GH, US). **Valle del Cauca** (16): Road Cali–Buenaventura, 20 km from Buenaventura, *Bischler 1446* (G); Río Naya, Puerto Merizalde, *Cuatrecasas 14078* (US); Río Yurumanguí, Veneral, *Cuatrecasas 15810* (F, US); Río Calima, La Trojita, *Cuatrecasas 16541* (F, US); Río Cajambre, Quebrada del Corosal, *Cuatrecasas 17730* (F, US), *17731* (UC); Bahía de Buenaventura, Quebrada de Aguadulce, *Cuatrecasas 19981* (F, US); Río Calima, Quebrada de la Brea, *Cuatrecasas 21199* (F); Córdoba, Dagua Valley, *Killip 5283* (NY, GH, US); Dagua Valley, along Río Calima, *Killip 11786* (GH, US); Agua

Clara, along Hwy. Buenaventura–Cali, *Killip & Cuatrecasas 38913* (F, US); ca. 18 km E of Buenaventura, *Killip & Garcia 33246* (COL, US); along Río Dagua, ca. 20 km E of Buenaventura, *Killip & Garcia 33320* (COL, US); Córdoba on Río Dagua, *Lehmann 3802* (B, G); Río Dagua, near Buenaventura, *Lehmann 4435* (US); Córdoba, Dagua Valley, *Pittier 529* (US).

10. **Cyclodium varians** (Fée) A. R. Smith, comb. nov. (Fig. 9, G–K).—*Nephrodium varians* Fée, Hist. Foug. Antill. (Mém. Fam. Foug. 11) 88, pl. 24, figure 2. 1866.—*Dryopteris varians* (Fée) Kuntze, Rev. Gen. Pl. 2:814. 1891.—*Stigmatopteris varians* (Fée) Alston, Kew Bull. 1932:309. 1932.—*Thelypteris varians* (Fée) Reed, Phytologia 17:323. 1968.—*Cyclodium varians* (Fée) Morton ex Vareschi, Fl. Venez. 1:368. 1969, nom. nud.—TYPE: Trinidad, 1864, *Germain s.n.* (P, not seen; cited by Christensen, 1913, as seen at Paris).

Rhizomes moderately long-creeping, with ca. 1 cm between stipe bases, ca. 5–8 mm diam., at apex with dark purplish-brown (with age nigrescent), shining, conform, linear scales 4–5 mm long, 0.3–0.5 mm wide, these ± entire or with a few marginal teeth, somewhat twisted at apex; fronds 45–75(105) cm long, somewhat dimorphous, the sterile ones shorter and with broader pinnae than the fertile; stipes equalling or slightly shorter than laminae, stramineous to usually tan, glabrescent, 2–5 mm diam., with persistent scales only at the very base; laminae to 50 cm long, 15–20 cm wide, with 9–17 pairs of lateral pinnae and a subconform, hastate terminal segment that tends to be more lobed and wider at base than the lateral pinnae; fertile pinnae subentire, sinuate, crenate, or shallowly lobed (lobes twice as wide as long), (5)10–15 cm long, 0.8–1.5 cm wide, the lowermost stalked to 5 mm, cuneate at the base, acute or acuminate at the tip; sterile or juvenile pinnae up to 12 cm long, 1.5–2.5 cm wide, often sharply serrate at the tip, truncate at the acroscopic base, cuneate basiscopically; veins anastomosing to form 1 or 2 series of areoles between costa and margin; costae and costules abaxially with scattered, ± appressed, reddish-brown, septate, hairlike scales up to 1 mm long; yellowish or translucent glandular hairs 0.1 mm long along adaxial rachis groove and abaxially at pinna bases, some similar glands also sometimes along costae abaxially; sori in (1)2–3 rows between costa and margin, discrete or occasionally subconfluent where veins join, with a large, reddish-brown, peltate, short-ciliate indusium up to 1.3 mm diam.

Distribution.—Known only from Trinidad (see Jenman, 1887) and Guyana (Fig. 8). A report from Surinam (Kramer, 1978, citing *Stahel BW 7218*) is based on a misidentification of *C. guianense*. Vareschi (1969, p. 368) listed it as to be expected in Venezuela, but I have seen no collections. All collections appear to have been made near sea level.

Discussion.—This is apparently an uncommon or rare species, and there are few recent collections. It has not been gathered since 1930, and I have seen only four collections from this century. Jenman (1909) reported it (as *Aspidium abbreviatum*, treated herein as a variety of *Cyclodium heterodon*, from the Brazilian coast) as "common on shady creeks on nearly all the rivers below the rapids," and "growing on stumps and roots of trees in the water." In many respects, it shows some morphological intermediacy between *C. meniscioides* (or perhaps *C. akawaiorum*) and *C. inerme*. One wonders whether it might not

have had an allopolyploid origin. This idea is reinforced by the unusual variability in cutting of some specimens (hence the specific epithet varians), even on the same frond. Jenman (1909), Christensen (1913), and Fée in his original description all commented on this variation, which can be readily seen in *Parker s.n.*, from Guyana, and *Purdie s.n.*, from Trinidad. In these two specimens, some pinnae are incised three-fourths or more of the distance to the costa, while other pinnae on the same frond are merely sinuate or shallowly crenate.

Little is known of the habitat of this species. Jenman (1909, p. 204) described it as "as often erect in growth as horizontal." The rather long-creeping rhizome shows little or no evidence of being buried in the soil. It seems possible that this species is a low-level hemiepiphyte, at least in some situations. Several collections show the fronds directed toward the rhizome apex, as if the rhizome axis were more or less vertical against or leaning upon other vegetation.

Additional collections: GUYANA. Essequibo River, Moraballi Creek, near Bartica, near 0 m, *Richards 22* (BM, K); Mazaruni River, 1899, *Jenman s.n.* (K, NY, US); Macourie Creek, Essequibo River, 1895, *Jenman s.n.* (NY); Essequibo, 1862, *Appun 27* (B, BM, P—not seen, W); Malali, Demerara River, *Jenman 6853* (GH), *s.n.* (K, NY); Demerara, 1830, *Parker s.n.* (K, 2 sheets); TRINIDAD. Aripo Savanna, *Britton & Britton 2941* (US); Aripo Savanna, *Othmer 116* (BM, M); Aripo, *Othmer 117* (M, 2 sheets); 1903, *Othmer s.n.* (P), *Prestoe s.n.* (BM); *Purdie s.n.* (BM); Aripo, Mar 1851, collector not legible (K); Trinidad Bot. Gard. Herb. 1225 (US).

EXCLUDED NAMES

Nephrodium acutum Hook., Sp. Fil. 4:147, pl. 271. 1862, non Presl (1825). ≡*Lastreopsis acuta* (Hook.) Tindale.—SYNTYPES: Brazil, *Sellow s.n.* (K); Peru, near Tarapoto, *Spruce 4662* (K). Implicitly lectotypified by Baker, Syn. Fil., ed. 2. 501. 1874, when he selected *Spruce 4662* (K) as type of *Nephrodium firmifolium Baker* [=*Cyclodium trianae* (Mett.) A. R. Smith]. Hooker's description of *Nephrodium acutum* is in agreement with the Sellow collection, while his illustration (pl. 271) is of the Spruce collection.

Dryopteris clypeata Maxon & Morton, Bull. Torrey Bot. Club 66:52. 1939. ≡ *Thelypteris clypeata* (Maxon & Morton) Kramer, Acta Bot. Neerl. 18: 141. 1969.—*Stigmatopteris clypeata* (Maxon & Morton) Lellinger, Proc. Biol. Soc. Washington 89:730. 1977.—TYPE: Panama, hills back of Puerto Obaldía, San Blas Coast, 50–200 m, *Pittier 4309* (US, 2 sheets). This is a member of subg. *Goniopteris* and is closely allied to *Thelypteris holodictya* Kramer from Surinam.

Aspidium extensum Fée, Gen. Fil. 294. 1852, non Blume (1828). = *Ctenitis melanosticta* (Kunze) Copel—Thought by Christensen (Ind. Fil. 73. 1905) to be the same as *Dryopteris trianae* [≡*Cyclodium trianae* (Mett.) A. R. Smith].

Nephrodium imrayanum Hook., Sp. Fil. 4:86, pl. 242A. 1862 = *Stigmatopteris rotundata* (Willd.) C. Chr.—The specimen (*Spruce 2153*) cited as this species by Fée (*Aspidium imrayanum* (Hook.) Fée, Crypt. Vasc. Brès. 1:133. 1869) is the type collection of *Polypodium sancti-gabrieli* Hook.

[= *Cyclodium guianense* (Klotzsch) A. R. Smith]. *Nephrodium imrayanum* is based on a collection from Dominica, *Imray* s.n. (K).

Literature Cited

Christensen, C. 1905–1906. *Index filicum*. Copenhagen: Hagerup.

———. 1909. On *Stigmatopteris*, a new genus of ferns with a review of its species. Bot. Tidsskr. 29:291–304.

———. 1913. A monograph of the genus *Dryopteris*. Part I. The tropical American pinnatifid-bipinnatifid species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Afd. (7)10:55–282.

———. 1920. A monograph of the genus *Dryopteris*. Part II. The tropical American bipinnate-decompound species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Afd. (8)6:1–132.

———. 1938. Filicinae. Pp. 522–550 in *Manual of pteridology*, ed. F. Verdoorn. The Hague: Nijhoff.

Copeland, E. B. 1947. *Genera filicum; the genera of ferns*. Waltham, Mass.: Chronica Botanica.

Hennipman, E. 1977. A monograph of the fern genus *Bolbitis* (Lomariopsidaceae). Leiden Bot. Series 2:1–331.

Holttum, R. E. 1984. Studies in the fern-genera allied to *Tectaria*, I. A commentary on recent schemes of classification. Brit. Fern Gaz. 12:313–319.

Hooker, W. J. 1862. *Species filicum*. London: William Pamplin.

———, and J. G. Baker. 1867. *Synopsis filicum*. London: Robert Hardwicke.

Jenman, G. S. 1887. The ferns of Trinidad. J. Bot. 1887:97–101.

———. 1909. *The ferns and fern allies of the British West Indies and Guiana*. [issued in parts, pt. 12, beginning p. 203, published in Feb 1908]. Ed. J. H. Hart. Trinidad: Government Printing Office, Port-of-Spain.

Kramer, K. U. 1978. The pteridophytes of Suriname. Uitgaven Natuurw. Studiekring Suriname Ned. Antillen 93:1–198.

Moore, T. 1857–1862. *Index Filicum*. London: Williams & Norgate.

Morton, C. V. 1939. On the genus *Cyclodium*. Bull. Torrey Bot. Club 66:47–52.

Pichi Sermolli, R. E. G. 1977. Tentamen pteridophytorum genera in taxonomicum ordinem redigendi. Webbia 31:313–512.

———. 1982. Third report of the Committee for Pteridophyta. Taxon 31:315–316.

Proctor, G. R. 1985. *Ferns of Jamaica*. London: British Museum (Natural History).

Silva Araujo, I. J. 1976. Polypodiaceae. P. 483 in IOPB chromosome number reports LIII, ed. A. Löve. Taxon 25:483–500.

Tryon, R. M. and A. F. Tryon. 1981. (581) Proposal to conserve *Stigmatopteris* C. Chr. 1909 against *Cyclodium* Presl, 1836, and *Peltochlaena* Fée, 1852 (Pteridophyta). Taxon 30:349–350.

———. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.

Walker, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Trans. Roy. Soc. Edinburgh 66:169–237.

———. 1973. Evidence from cytology in the classification of ferns. Pp. 91–110 in *The phylogeny and classification of the ferns*, ed. A. C. Jermy et al. J. Linn. Soc., Bot. 67, Suppl. 1.

Appendix: List of Exsiccata

The following is a complete list of all numbered collections seen; unnumbered collections are not listed. Parenthetical numbers refer to numbers assigned taxa in the taxonomic treatment. For mixed gatherings all identifications are listed. Type collections are indicated by a boldface **T**; they are not necessarily the type of the accepted name.

Alverson et al. 140 (9b); *143* (9b). *Appun 27* (10); *166* (6a); *1176* (6c). *Argent et al. 6455* (6a). *Austin et al. 7214* (6a). *Aymard & Stergios 358* (6a). *Aymard et al. 954* (5); *955* (5); *4196* (6c).

Balansa 325 (6a). *Benoist 715* (6a). *Berlin 949* (6a). *Bernardi 2957* (5); *7245* (5); *7283* (6a). *Bierhorst*

S70 (6a). *Billiet & Jadin 1677* (5); *1923* (5). *Bischler 1446* (9b); *2461* (2); *2592* (2); *2628* (3); *Black 54-16942* (6a). *Blanchet 2208* (4b); *2251* (4b); *2468* (4b); *2486* (4b); *2519* (6a). *Boom & Mori 1574* (3); *1894* (6a); *1990* (3). *Boom et al. 762* (4b). *Brade 15360* (6a). *Brade & Appaini 18130* (4a). *Brandbyge & Asanza 31930* (9a). *Britton & Britton 2941* (10). *Broadway 5758* (6a); *6009* (6a).

Cardona 1641 (3). *Conant 914* (6a); *1007* (6a); *1008* (6a); *1009* (6a). *Cowan & Soderstrom 2157* (5). *Cremers 5389* (5); *5390* (5); *5567* (6a); *5696* (5); *5697* (5); *5766* (5); *6084* (6a); *6126* (3); *6389* (3); *6559* (6a); *6608* (3); *7019* (6a); *7370* (3); *7411* (5); *7724* (5); *7828* (5). *Croat 18383* (6a); *18464* (6a); *18579* (6a); *18710* (6a); *18771* (6a); *20311* (6a); *20967* (6a); *27128* (9a). *Croizat 782* (3). *Cuarto 154* (4a). *Cuatrecasas 6996* (6a); *10563* (6a); *14078* (9b); *14130* (9b); *15810* (9b); *16541* (9b); *17730* (9b); *17731* (9b); *19981* (9b); *21199* (9b); *21421* (9b).

Daniëls & Jonker 744 (3); *852* (3); *1259* (6a); *1300* (6a). *Davidse & González 14294* (6a); *21735* (3). *Davis 180* (3). *Dawson 14893* (6a). *de Granville C.123* (3); *C.155* (5); *239* (5); *560* (5); *777* (5); *886* (5); *1445* (6a); *1753* (5); *1761* (6a); *1860* (3); *2123* (3); *2580* (5); *2586* (7-**T**); *2626* (5); *2752* (3); *2894* (6a); *3078* (6a); *3093* (3); *3288* (6a); *3530* (5); *4149* (3); *4730* (6a); *5243* (5); *5301* (3); *5302* (5); *5481* (6a); *5888* (5); *6455* (5). *de la Cruz 1174* (6a); *1177* (6a); *1921* (6a); *3119* (6a); *3488* (6a); *3844* (6a); *3847* (6a); *4302* (6a). *Delascio et al. 9604* (5). *de Nevers & Herrera 4001* (9b). *de Santos et al. R.1486* (6a). *Duarte 6756* (4b). *Duke 15522* (9b).

Edwards 1136 (5). *Eiten et al. 2125* (6a). *Ellenberg 2803* (6a); *2868* (6a); *2870* (6a). *Engel 231* (3). *Erskine 204* (6a). *Every & Burgess 52* (6a); *258* (6a). *Ewan 16861* (9b).

Fanshawe 4902 (5). *Fendler 97* (3); *3145* (3). *Fiebrig 4984* (6a); *6344* (6a). *Florschütz & Florschütz 1386* (5). *Florschütz & Maas 2975* (3); *2983* (6a); *3047* (3). *Foldats 2720* (6a). *Forbe 1080* (6a). *Forero & Jaramillo 2528* (9b); *4450* (9b). *Forero et al. 1342* (9b); *5269* (9b). *Fosberg 29222* (6a). *Froes 26025* (5).

García-Barriga 14336 (6a); *14347* (6a). *Gardner 1218* (4b/4 × 6?); *2759* (6a); *5671* (4a). *Geijskes 1012* (3). *Gentry & Renteria 24057* (9b); *24421* (9b). *Gentry et al. 15824* (6a). *Glaziou 5392* (4a); *7322* (6a); *10180* (6a); *12373* (5); *12374* (6c-**T**). *Gleason 183* (6a). *Glocker 10* (6a). *Gonggrijp & Stahel 601* (5); *974* [= *BW974*] (6a). *Gottsberger 34-11067* (6a). *Guppy 6095* (6a); *6135* (5); *6195* (5); *6212* (5); *6213* (5); *6249* (6a); *6250* (6a); *7418* (6a).

Harley 17173 (4b). *Harling & Andersson 17387* (9a); *17534* (9a); *17617* (9a). *Hart 6791* (6a). *Hassler 4783* (6a); *9049* (6a); *10131* (6a); *10131a* (6a); *11625* (6a). *Haught 1353* (2-**T**). *Hertel et al. 15280* (6a). *Hitchcock 17124* (6a). *Holm-Nielsen et al. 21985* (9a); *21987* (9a); *22029* (9a); *22061* (9a). *Holst & van der Werff 2573* (6a). *Holt & Blake 602* (3). *Hombersley 132* (6a). *Hostmann 15* (5-**T**). *Huber 753* (5).

Irwin & Soderstrom 5739 (6a); *5881* (6a). *Irwin & Westra 47168* (6a). *Irwin et al. 10139* (6a); *16303* (6a); *54523* (5); *54923* (3).

Jaramillo & Coello 3648 (9a). *Jenman 1481* (5); *1419* (5); *1600* (6a); *4213* (5); *6850* (6a); *6853* (10).

Killip 5283 (9b); *11786* (9b); *35084* (9b); *35287* (9b-**T**). *Killip & Cuatrecasas 38913* (9b). *Killip & Garcia 33172* (9b); *33246* (9b); *33320* (9b); *33622* (9b). *Killip & Smith 26165* (6a); *26566* (6a); *28540* (9a); *30316* (6a); *30331* (6a); *30335* (5). *Klug 241* (6a); *396* (6a). *Kramer 2364* (5). *Kramer & Hekking 2401* (6a); *3075* (6a); *3120* (5). *Kramer et al. 3254* (6a). *Krukoff 4924* (6a).

Lanjouw & Lindeman 543 (5); *2364* (5); *2389* (6a); *2457* (5); *2517* (5); *2720* (5); *2849* (6a). *Lehmann B.T.443* (9b); *3802* (9b); *4435* (9b). *Lellinger & de la Sota 82* (9b); *211* (9b); *253* (9b); *343* (9b); *417* (9b); *478* (9b); *562* (9b); *590* (9b). *Leng 103* (5); *202* (5). *Leprieur 27* (6a); *41* (3); *42* (5); *104* (5); *131* (6a); *188* (5-**T**); *190* (3); *270* (6a). *Liesner 3466* (6a); *3535* (6a); *16307* (3); *16345* (6a); *16354* (6a). *Liesner & González 10375* (3); *10383* (6a); *13307* (2). *Lindeman 3997* (5); *4238* (6a); *4752* (6a); *4946* (6a). *Lindeman et al. 143* (5); *154* (5); *227* (5); *228* (5); *305* (5); *356* (5); *412* (5); *632* (5). *Linder 27* (6a); *124* (6a). *Lleras* et al. *P17441* (6a). *Löfgren 255* (6a). *Luetzelburg 21267* (6a); *22064* (6a); *23656* (6a). *Luschnath 119* (4b).

Maas & Maas 2484 (6a). *Maas & Tawjoeran 10765* (6a); *10766* (6a); *10923* [= *LBB 10923*] (5). *Maas et al. 2539* (5); *2669* (6a); *3957* (5); *3990* (5); *4436* (5); *5891* (5). *Macedo 1839* (6a); *3370* (6a). *Maguire 24815* (3). *Maguire & Fanshawe 22821* (6a). *Maguire & Cowan 39295* (6a). *Maguire & Fanshawe 23525* (5). *Maguire & Stahel 24468* (3). *Maguire et al. 36875* (3); *36883* (3); *37391* (3); *37457* (5); *39064* (3); *39066* (5); *39071* (5); *39073* (5); *54231* (5); *54285* (5); *60279* (3). *Martius* [Fl. Bras.] *325* (4b); *326* (4b). *Mathias & Taylor 5138* (6a). *Melinon 376* (5). *Mell & Mell 205* (6a). *Mexia 6042* (5); *6193a* (6a); *6894* (9a); *8452* (9a). *Meyer 6219* (6a); *6280* (6a). *Monsalve 433* (9b). *Moran 3577* (6a); *3629* (6a). *Mori et al. 11351* (4b). *Mosén 3941* (6a). *Moss 26* (6a). *Müller & Müller 2526* (6a).

Oldeman *T415* (6a). *Øllgaard et al. 34511* (6a); *34528* (9a); *34742* (6a); *35360* (6a). *Othmer 116* (10); *117* (6a/10).

Pennell 3723 (6b-**T**). *Persaud 110* (1). *Philcox et al. 3710* (6a); *3911* (6a). *Pinas 8354* [= *LBB 8354*] (6a). *Pittier 529* (9b); *4413* (9b); *4415* (9b). *Plowman 2452* (6a). *Plowman et al. 9532* (5). *Poole 1822* (5); *1922* (3); *1930* (3); *1958* (3). *Potter 5281* (6a). *Prance & Pennington 1825* (3). *Prance et al. 9452* (3); *15945* (6a); *19947* (3); *22686* (3); *59224* (6a). *Prestoe 1232* (3). *Pulle 386* (6a).

Ramos & Sousa 157 (6a). *Ratter et al. 1022* (6a); *R2134* (6a). *Richards 15* (6a); *22* (10); *799* (6a); *6591* (6a); *6625* (6a). *Richards et al. R504* (6a). *Riedel 43* (4b); *47* (4b); *82* (6a). *Ruiz-Terán & López-Palacios 11473* (1). *Rusby & Squires 377* (6a); *379* (6a).

Sandwith 1473 (5). *Sastre 3017* (6a). *Schomburgk 25* (3/5); *135* (5); *316* (6a); *1157* (3-**T**); *16174* (6a). *Schubert 2224* (5). *Schultes 5659* (3); *5661* (3). *Schultes & López 9427* (3). *Schultes & Cabrera 13474* (6a); *13928* (3); *16647* (6a); *17354* (6a); *19329* (3). *Schultes & Wessels Boer 10163* (3); *10175* (3). *Schulz 8043* (5); *8695* [= *LBB 8695*] (6a); *9943* [= *LBB 9943*] (6a). *Schunke 5610* (6a). *Schwacke 200* (6a). *Smith, A. C. 2745* (3); *2896* (5). *Soejarto & Cardozo 718* (6a); *719* (6a); *740* (6a). *Solomon 3565* (6a); *3573* (6a); *6186* (6a). *Spruce 25* (6a); *26* (6a); *36* (5-**T**); *379a* (6a); *379b* (6a); *2153* (3-**T**); *2159* (3); *2775* (6a); *2776* (6a); *4662* (9a-**T**); *4689* (6a). *Stahel 463* [= *BW 7218*] (3); *496* [= *BW 7202*] (6a). *Stahel & Gonggrijp 601* [*BW 601*]; *2642* [= *BW 2642*]. *Steyermark 57958* (3); *58077* (3); *87654* (3); *88601* (5); *89104* (5); *89529* (5); *90656* (3). *Steyermark & Bunting 102582* (3). *Steyermark & Nilsson 9* (1); *124* (1). *Steyermark et al. 92644* (1); *122169* (5); *130318* (5).

Thomas & Plowman 3157 (6a). *Tillett et al. 44942* (5). *Tresling B496* (5). *Triana 32* (9a-**T**). *Tryon & Tryon 5205.5* (6a). *Tutin 420* (6a).

Ulbricht 121 (6a). *Ule 9128* (6a).

van der Werff 604 (6a). *van der Werff & González 5207* (6a). *van der Werff & van Hardeveld 6986* (9b). *van Donselaar 3740* (6a); *Vareschi 6904* (6a); *Vargas* [*Univ. Cuzco 11476*] (6a). *Versteeg 308* (6a).

Warrington et al. K.E.R.76 (1-**T**); *K.E.R.167* (6c); *K.E.R.1207* (1). *Williams, L. 11381* (3). *Williams, R. S. 1191* (6a); *1202* (6a). *Wullschlaegel 1710* (3). *Wurdack & Monachino 41294* (6a).

Shorter Note

***Cystopteris tennesseensis* in West Virginia.**—*Cystopteris tennesseensis* Shaver (Tennessee Bladder-fern) is a fertile allopolyploid that apparently originated from an ancestral cross between *C. bulbifera* (L.) Bernh. and *C. protrusa* (Weath.) Blasdell. The relationships of *C. tennesseensis* to other *Cystopteris* species have recently been described by Haufler (Proc. Roy. Soc. Edinb. 86B:315–323, 1985). Tennessee Bladder-fern is widely distributed over the eastern and central United States from Maryland, Virginia, North Carolina, and Georgia, west to Kansas and Oklahoma (Blasdell, Mem. Torrey Bot. Club 21:1–102, 1963; Cranfill, *Ferns and fern allies of Kentucky*, 1980; Moran, Amer. Fern J. 72:93–95, 1982). While *C. tenneseensis* seems to be rare or local in the easternmost part of its range, it is difficult to assess accurately its total geographic distribution and frequency. Many regional manuals do not include this fern or mention it only peripherally. Tennessee Bladder-fern also is under-reported since it may grow in mixed populations with *C. bulbifera* or the Cliff Fragile-fern, *Cystopteris tenuis* (Michx.) Desv. This latter species has long been known as *C. fragilis* (L.) Bernh. var. *mackayi* Lawson (Moran, Castanea 48:224–229, 1983). Collectors may fail to survey the entire population and thus collect only one of the species present. The problem is further compounded by hybridization between species (Moran, 1983). Consequently, *C. tennesseensis* often is overlooked or misidentified.

Tennessee Bladder-fern has not previously been reported from West Virginia, though Strausbaugh and Core (*Flora of West Virginia*, p. 20, 1978) suggested that it likely would be found in the state. *Cystopteris tennesseensis* has been collected from five counties in West Virginia. The specimens examined are listed below.

West Virginia: **Brooke Co.**: Shaley talus, Cross Creek, S of McKinleyville, 17 Oct 1983, *Cusick 23209* (NCU, WVA). **Grant Co.**: Greenland Gap Rd, E of Scherr, 30 July 1978, *Rader 2091 & White* (WVA). **Pleasants Co.**: Near St. Marys, 8 May 1937, *Brooks & Margolin s.n.* (WVA); 1 mi S of Eureka, 1 June 1945, *Bartholomew s.n.* (WVA); sandstone, Middle Island Creek, E of St. Marys, 6 Oct 1983, *Cusick 23180 & Ortt* (MICH). **Putnam Co.**: Bridge Creek Rd., 10 June 1983, *Denison s.n.* (WVA). **Tucker Co.**: Sandstone cliff, Otter Creek, base of Green Mountain, 12 Oct 1957, *Rossbach 1170* (WVA).

West Virginia is a logical part of the total range of *C. tennesseensis*. Among adjacent states this species is frequent only in Kentucky and Ohio. The only known Virginian population is in Montgomery County (Moran, Jeffersonia 13: 30–34, 1982). The species has been collected once in Frederick County, Maryland (Wagner, Amer. Fern J. 34:125–127, 1944). The North Carolina populations of Tennessee Bladder-fern represent the extreme southeastern limit of its known distribution. These sites, in Craven and Jones counties, are the only known occurrences on the Atlantic Coastal Plain (Blasdell, 1963). Reports of *C. tennesseensis* from Pennsylvania (Wherry et al., *Atlas of the flora of Pennsylvania*, p. 10, 1979) need confirmation; they may be based in part upon *C. laurentiana* (*C. bulbifera* × *fragilis*). Tennessee Bladder-fern should be sought in southcentral and southwest Pennsylvania and in western Maryland, as well as in additional West Virginian counties.

Cystopteris tennesseensis is easily mistaken for its putative parents. Most of

the West Virginian specimens examined had been misidentified as *C. bulbifera*. Moran (1982) reported similar difficulties in identification of *C. tennesseensis* in Illinois. The first two Pleasants County collections cited above are the basis for the Strausbaugh and Core report of *C. bulbifera* in that county. These two species grow in similar rocky habitats. They seldom are found on stream terraces or in deep soils, the typical habitat of *C. protrusa*. *Cystopteris tennesseensis* often grows in more xeric situations than *C. bulbifera*. Although *C. tennesseensis* is most frequent and abundant on limestone in most of its range, in Ohio and West Virginia it is found variously on limestone, dolomite, shale, and sandstone. Substrate is recorded for only three of the eight West Virginia collections cited above.

Tennessee Bladder-fern apparently is rare or local in West Virginia. However, more thorough collecting is needed to determine accurately its status in the flora.

I thank Dr. Roy B. Clarkson and Linda Rader, Department of Biology, West Virginia University, for their courtesy and cooperation. Robbin Moran, Illinois Natural History Survey, Champaign, kindly shared with me his thoughts on *Cystopteris*. Marilyn Ortt, Ohio Department of Natural Resources, provided valuable field assistance.—ALLISON W. CUSICK, Division of Natural Areas & Preserves, Ohio Department of Natural Resources, Fountain Square, Columbus, OH 43224.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

AMERICAN FERN JOURNAL

Volume 76

Number 3

July–September 1986

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

The Use of Fern Herbarium Specimens in Biosystematic Research: Introduction
Michael D. Windham **101**

Active Enzymes from Herbarium Specimens: Electrophoresis as an Afterthought
Thomas A. Ranker and *Charles R. Werth* **102**

Biosystematic Uses of Fern Gametophytes Derived from Herbarium Specimens
Michael D. Windham and *Christopher H. Haufler* **114**

Detecting Abortive Spores in Herbarium Specimens of Sterile Hybrids
W. H. Wagner Jr., F. S. Wagner, and *W. Carl Taylor* **129**

Factors Affecting Prolonged Spore Viability in Herbarium Collections of Three Species of *Pellaea*
Michael D. Windham, Paul G. Wolf, and *Thomas A. Ranker* **141**

Systematic Inferences from Spore and Stomate Size in the Ferns
David S. Barrington, Cathy A. Paris, and *Thomas A. Ranker* **149**

Information for Authors **160**

The American Fern Society

Council for 1986

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *President*
JUDITH E. SKOG, Biology Dept., George Mason University, Fairfax, VA 22030. *Vice-President*
W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*
JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*
DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*
JAMES D. MONTGOMERY, Ecology III, R.D. 1, Berwick, PA 18603. *Back Issues Curator*
ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*
DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*
DENNIS Wm. STEVENSON, Dept. of Biological Sciences, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH ..... Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47401
CHRISTOPHER HAUFLER ..... Dept. of Botany, University of Kansas, Lawrence, KS 66045
DAVID B. LELLINGER ..... U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560
TERRY R. WEBSTER .. Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editors (Dennis Wm. and Jan Wassmer Stevenson) welcome contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 76(3):101 (1986)

The Use of Fern Herbarium Specimens in Biosystematic Research: Introduction

MICHAEL D. WINDHAM
Department of Botany, University of Kansas, Lawrence, Kansas 66045

Dried specimens have been an indispensable part of botanical science since the first half of the sixteenth century (Morton, 1981). This is especially true for descriptive botany and classical taxonomy, which provide the foundation for modern studies of plant evolution. In recent years, the focus of evolutionary investigations has shifted from the relatively stable end products (species) to the dynamic process itself. This has led to the belief that the most accurate reconstruction of relationships is achieved through an amalgamation of morphological, genetic, biochemical, physiological, and ecological data. As a result, classical morphological taxonomy is being supplanted by biosystematics, an integrated approach that emphasizes the use of live plants (Solbrig, 1970).

As the popularity of biosystematics has increased, the perceived importance of herbarium specimens has declined. Lagging interest in preserved materials has, in turn, led to a restructuring of budgetary priorities within the biological sciences. Many herbaria show signs of serious financial neglect, including curtailment of hours (e.g., CAN; see Anon., 1985), reduced availability of loans (e.g., BUT; see Holmgren et al., 1981), and lower rates of specimen accession. This is an alarming trend that, if continued, will eventually undercut public support for botanical research. Admittedly, there are many facets to the budgetary crisis in botany, but the shift from classical taxonomy to biosystematics should not be a contributing factor. As will be shown in the following articles, most of the techniques commonly used in biosystematic research can be applied successfully to herbarium specimens. Many of these methods are non-destructive (i.e., acceptable to curators), and they can yield valuble information on relationships, breeding systems, hybridization, and other aspects of evolution. In the papers that follow, we begin to explore the potential of herbarium collections for biosystematic research among pteridophytes.

LITERATURE CITED

ANONYMOUS. 1985. Major cutbacks at Canadian National Museum of Natural Sciences. Assoc. Syst. Coll. Newsl. 13:44.

HOLMGREN, P., W. KEUKEN, and E. SCHOFIELD. 1981. Index herbariorum. Part I. 7th ed. Regnum Veg. 106:1–452.

MORTON, A. 1981. *History of botanical science*. London: Academic Press.

SOLBRIG, O. 1970. *Principles and methods of plant biosystematics*. Toronto: Macmillan Co.

MISSOURI BOTANICAL

NOV 6 1986

American Fern Journal 76(3):102–113 (1986)

Active Enzymes from Herbarium Specimens: Electrophoresis as an Afterthought

THOMAS A. RANKER and CHARLES R. WERTH
Department of Botany, University of Kansas, Lawrence, Kansas 66045

Electrophoretic analyses of enzyme variation in plants routinely employ fresh leaf tissue. Extracts of such material are analyzed immediately or the leaves or extracts are preserved through freezing or lyophilization. The possibility that active enzymes may be obtained from dried pressed specimens for electrophoretic analyses has not been considered by population biologists or systematists. In the present study, however, we demonstrate that, in certain cases, electrophoretic investigations of pteridophytes can be extended to include dried sporophytic material. This expansion of the utility of pressed specimens from herbaria greatly enhances the potential of such material to contribute to biosystematic research.

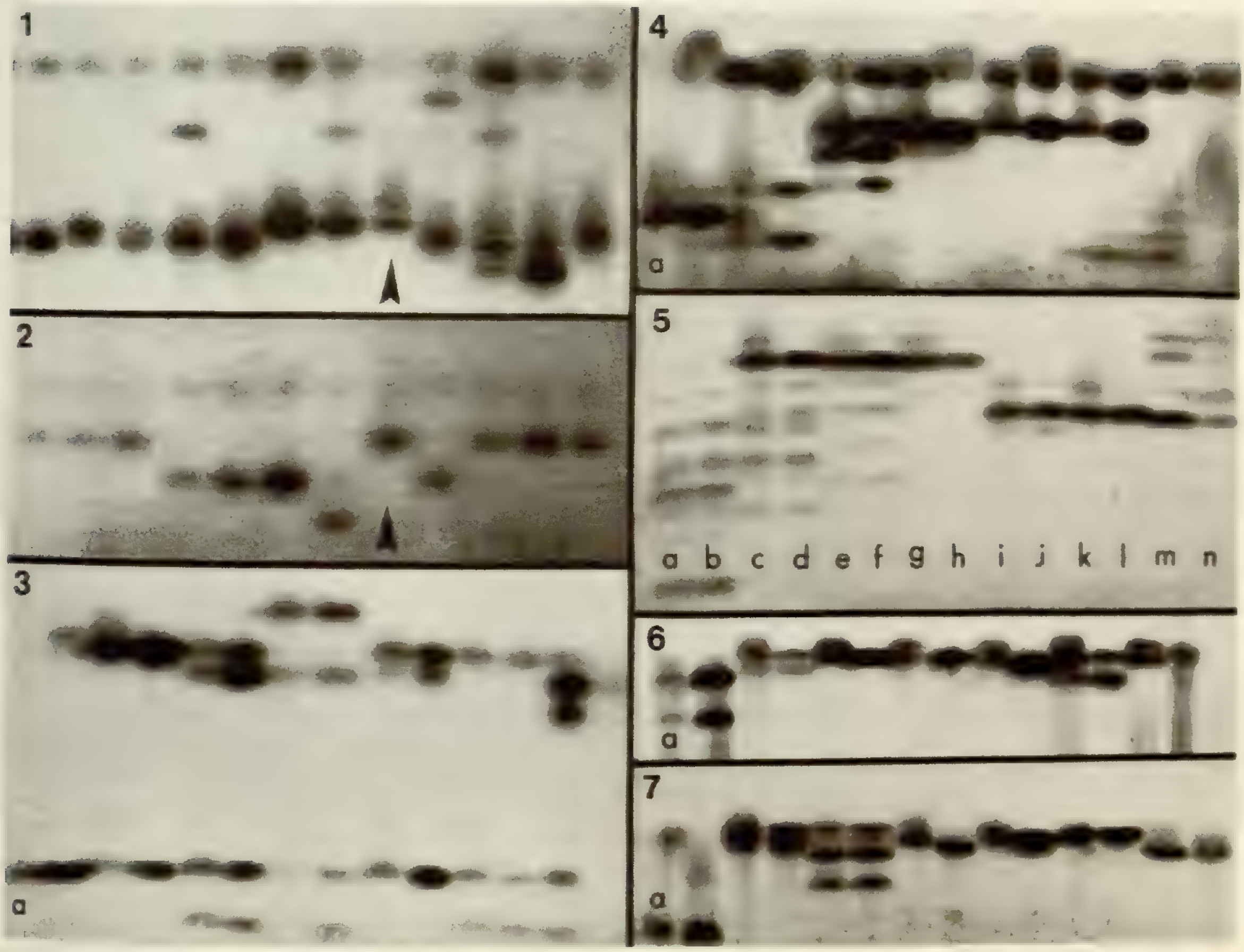
Numerous studies on the effects of dehydration on the anatomy, biochemistry, and physiology of desiccation-tolerant and desiccation-intolerant plant species have been reported (e.g., see reviews by Iljin, 1957; Todd, 1972; Crowe & Clegg, 1978; Bewley, 1979; Bewley & Krochko, 1982). These effects are complex, vary among species, and often depend upon the particular conditions of dehydration and rehydration, even within a taxon. In general, however, the studies have shown that desiccation-tolerant plants are capable of quickly restoring photosynthesis and respiration upon rehydration and, under certain conditions, desiccation-intolerant species may also resume these functions. Thus, it has been established that some dehydrated plants can preserve enzymes that are capable of continuing physiological activity upon rehydration.

The present study was initiated following the observation that electrophoretic expression of enzymes had been obtained from leaf tissue of *Asplenium platyneuron* plants that inadvertently had been allowed to desiccate in plastic bags at 4°C (Figs. 1, 2). This led to a more extensive survey of dried pressed material of varying ages and from a variety of taxa.

MATERIALS AND METHODS

Small quantities of leaf or rhizome material (5–15 mg) were removed from pressed specimens (see Table 1 for location of vouchers) and ground in the phosphate crushing buffer of Soltis et al. (1983). The grinding was done in the wells of porcelain spot plates using the rounded ends of small test tubes as pestles. The exudate was absorbed onto rectangular wicks of Whatman 3 MM chromatography paper. The wicks were inserted immediately into 12.8% starch gels for electrophoresis.

Enzymes were surveyed using buffer systems 6, 8, and 11 as outlined by Soltis et al. (1983; modified in Haufler, 1985) and the continuous tris-citrate buffer (gel



FIGS. 1–7. Photographs of gels. Anode is at the top of each figure. FIGS. 1, 2. Electrophoretic banding patterns of *Asplenium platyneuron* sporophytes. Bands at and to the right of the arrows are from desiccated leaves; all others are from fresh leaves. FIG. 1. PGI. FIG. 2. PGM. FIGS. 3–7. Comparisons of electrophoretic banding patterns from fresh and dry sporophytic material, arranged in pairs of dry followed by fresh of same individual, except for last pair (m–n) which is fresh-dry. Lanes in all five figures correspond to those labeled in Fig. 5. Lane a is marked for reference in all figures. a–b: *Dryopteris carthusiana* (dry material 0.2 yrs old); c–d, e–f: *Woodsia oregana* (0.5); g–h, i–j, k–l: *W. mexicana* (0.5); m–n: *W. obtusa* (0.5). FIG. 3. PGM. FIG. 4. PGI. FIG. 5. TPI. FIG. 6. LAP. FIG. 7. 6-PGD.

pH 8.0) of Selander et al. (1971). Esterase (EST), amino aspartate transaminase (AAT), and phosphoglucomutase (PGM) were assayed on system 6; hexokinase (HK) and leucine aminopeptidase (LAP) on system 8; acid phosphatase (AcPH) on system 11 or the tris-citrate; isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and shikimate dehydrogenase (SkDH) on system 11; phosphoglucose isomerase (PGI) and triose-phosphate isomerase (TPI) on systems 6 and 8. Standard staining schedules were employed (e.g., see Soltis et al., 1983; Werth, 1985).

RESULTS AND DISCUSSION

The results are summarized in Table 1. They show that while active enzymes were obtained from a variety of pressed materials, there was considerable variation in the quality of the results according to the species, age of material, and the enzyme system assayed. Most specimens exhibited some activity for at least

several enzymes. In a number of cases, however, discrete bands could not be resolved. In such cases the enzyme molecules may have been so degraded that attempts to obtain greater resolution by the use of different electrophoretic conditions may not be productive. Thus, the most promising cases for further investigation are those for which discrete bands could be identified. Many such cases were observed (Table 1) and some will be discussed in greater detail below. In many of the examples, bands could be identified that were not clearly resolved or which seemed to be accompanied by more bands than would be expected at a locus, given a knowledge of enzyme structure (Gottlieb, 1981) and intracellular compartmentalization of isozymes (Gastony & Darrow, 1983; Soltis, 1986). Extra bands may be due to post-translational modification, inappropriate treatment of material causing the production of artifacts (Harris & Hopkinson, 1976), or changes in molecular conformation related to desiccation (Todd, 1972). These "ghost" bands sometimes may be eliminated or reduced by changes in experimental technique such as the use of different gel and electrode buffers or of a different grinding buffer (Harris & Hopkinson, 1976; Soltis et al., 1980). Banding patterns from dried specimens that are not completely resolvable may still be of value if they can be interpreted when directly compared to results from living plants of the same species (see below).

The ability to preserve enzyme integrity for relatively short periods does not seem to be related to the living plant's ability to tolerate desiccation. With one exception (*Bommeria pedata*), all specimens less than one year old showed resolvable enzymatic activity at most or all loci assayed. These included species of *Dryopteris* (Figs. 3–7), *Hemionitis, Lycopodium, Polypodium,* and *Woodsia.* The best overall results among these taxa were obtained from the *Woodsia* specimens (Figs. 3–7). These species grow in mesic habitats and have delicate, easily dehydrated leaves, yet clearly resolved isozyme bands were obtained from six-month-old pressed leaves.

Isozymic expression can often be obtained from pressed material that is comparable to that from live plants. Because the *Woodsia* leaves were collected from plants under cultivation, we had the opportunity to compare isozymic patterns from dry material directly with those of fresh leaves of the same individuals. Similarly, for three species of *Pellaea,* comparisons could be made to live gametophytes grown from viable spores obtained from the same herbarium specimens (see Windham & Haufler, 1986; Windham et al., 1986). In this way we could check for qualitative and quantitative changes in electrophoretic expression upon dehydration. It can be seen from Figs. 3–7 that for most enzymes there were only slight decreases in the quality of the bands from the dried material and that the quantity of bands remained the same. One notable exception to the latter in *Woodsia* was for LAP where all of the dried specimens expressed an extra band not found in living plants (Fig. 6). Results comparable to those from live gametophytes were obtained in dried leaves of two-year-old *P. ternifolia* and eight-year-old *P. wrightiana* for LAP, TPI, 6-PGD, SkDH, IDH, and HK. Partial or complete loss of expression was observed in PGI, MDH, and PGM. Dried 23-year-old *P. truncata* showed resolvable bands only for TPI; however, a three-year-old specimen of this species had clear bands at most loci examined.

These results are similar to those obtained from wilted wheat leaves (Todd, 1972) where some isozymes showed only qualitative changes upon wilting while others showed both qualitative and quantitative shifts in expression. These changes apparently are due, directly or indirectly, to the effects of desiccation (Todd, 1972; Bewley & Krochko, 1982). These results have two important implications: 1) some enzyme systems may not be scored reliably using pressed specimens, depending upon the purpose of the study; and, 2) results from pressed material should be compared to those from live material of the same species for accurate assessment of banding patterns.

The ability to preserve enzyme activity beyond about a year may be related to the desiccation tolerance of a species and/or to the particular conditions of drying or storage. For example, a two-year-old specimen of *Polypodium polypodioides* (Table 1) produced resolvable bands at all loci examined from both rhizomes and leaves. These results are not surprising given the recurrence of dehydrating conditions in the life history of this epiphyte. Stuart (1968) showed that leaves of this species could lose up to 97% of their water content and resume normal photosynthesis and respiration upon rehydration. In contrast, though, a conspecific one-year-old specimen (Table 1) exhibited clear bands for all loci from rhizome extracts but for only three of eight enzyme systems from the leaves. Both of our specimens were collected as green, apparently viable, individuals. Thus, there may have been differences in the conditions of drying (see below) or storage between these specimens that affected enzyme activity. More data are needed to assess accurately the effects of these variables.

In this study particular attention was given to the ability of *Selaginella* species to preserve active enzymes since many species of this genus are known for their extreme tolerance of desiccation. An initial assay indicated that specimens of xerophytic species were more likely to be of value electrophoretically than were those of mesophytes (e.g., compare *S. apoda*, a mesophytic species, with other taxa, all xerophytes; Table 1); hence, subsequent analyses only included representatives of the former. The oldest specimen of *Selaginella* to provide evidence of enzymatic activity was one of *S. wallacei*, collected 64 years ago. Specimens of other species up to about 25 years old provided resolved banding patterns for most loci. Several species were sampled across decades (most thoroughly in *S. densa* and *S. rupestris*) over which a general increase in activity and resolution was observed with a decrease in age (Figs. 8–13).

Previous studies have reported on the ability of desiccated *Selaginella* specimens to resume growth and various physiological functions. Tryon (1955) reported that a six-month-old dry specimen of *S. densa* var. *densa* resumed growth upon rehydration. Webster and Steeves (1964) found that a mat of *S. densa* stored dry for two years and nine months showed signs of growth when watered for several weeks. Eickmeier and coworkers (Eickmeier, 1979, 1980, 1983; Bergtrom et al., 1982) have done extensive work on photosynthetic recovery of desiccated *S. lepidophylla*. They have shown that individuals that have been desiccated for at least two years may fully recover photosynthetic and respiratory function upon rehydration. Uphof (1920) studied the anatomy of several xerophytic species of *Selaginella* and described a number of anatomical features that may contrib-

TABLE 1. Summary of Electrophoretic Analyses of Dried Plant Specimens.

Taxon	Age of material in years	Source[1]		Enzyme system AAT	AcPH	EST
Adiantum concinnum	1	Ranker 786			+++	
Asplenium bradleyi	4	Magrath et al. 12156			++	
Asplenium bradleyi	17	Matthews et al. s.n.			++	
A. monanthes	1	Ranker 790			++	
A. montanum	5	Werth 81M18			+++	
A. montanum	13	Lafferty 1306			+++	
A. pinnatifidum	4	Magrath et al. 12147			++	
A. pinnatifidum	31	McGregor 3719			++	
A. platyneuron	2	McGregor 34352			+++	
A. platyneuron	17	Johnson 1306			+++	
A. rhizophyllum	5	Brooks & Harris 15155			+++	
A. rhizophyllum	5	Werth 81S19			+++	
Aspidotis densa	1.5	Ranker et al. 756			+++	
*Bommeria pedata**	0.5	Ranker et al. 801		−	−	−
Cheilanthes parryi	2.5	Ranker & Simmons 794			+++	
C. eatonii	38	Weber 3290				−
C. eatonii	24	Wilson 3962				−
C. eatonii	15	Stephens & Brooks 43000				−
C. wootonii	18	Brooks 493				−
*Cystopteris diaphana**	1	Ranker 780		−	+++	−
Dryopteris carthusiana	0.2	Windham et al. 688				
D. marginalis	0.5	Werth KF			++	
*Hemionitis elegans**	0.5	Ranker et al. 803		++	−	+++
H. palmata	1	Ranker et al. 727			++	
Lycopodium dendroideum	0.5	Ranker 838			++	
L. lucidulum	0.5	Ranker 837			+	
L. reflexum	1	Ranker et al. 769		−	−	−
L. sp.	1	Ranker et al. 783		−	−	−
L. sabinifolium	0.5	Ranker et al. 840-2		−	+	++
*Notholaena formosa***	1.5	Windham et al. 539				
*N. limitanea***	1.5	Windham et al. 575				
*N. pallens***	1.5	Windham et al. 527				
Pellaea atropurpurea	39	Reed s.n.				−
P. atropurpurea	9	Brooks & Hauser 12238				−
P. mucronata	28	Hartman 1281				−
*P. ternifolia***	2	Weber & Bajakiewicz 16507 (COLO)	rh[2] lf[2]			
*P. truncata***	23	Martin 5146 (UNM)	rh lf			
*P. wrightiana**	19	Bozeman et al. 45152				+
*P. wrightiana***	8	Reeves et al. 6340 (ASU)	rh lf			
Pityrogramma tartarea	1	Ranker 770		−	−	−
P. triangularis	1.5	Ranker & Wolf 754			+++	
Polypodium adelphum	1	Ranker 815		−	+++	−
*P. aureum**	2	Ranker et al. 717	rh lf			
*P. californicum**	5	Ranker & Artus 96		−	+++	−
P. furfuraceum	2	Ranker et al. 720	rh lf			
P. glycyrrhiza	5	Ranker & Artus 203 & 267	rh lf		+++ +++	

TABLE 1. Extended.

Enzyme system								
HK	IDH	LAP	MDH	6-PGD	PGI	PGM	SkDH	TPI
			++	+	−	−	−	
	−		−			−	−	
	−		−			−	−	
			++	+	++	++	+	
	−		+			−	+	
	−		−			−		
	−		−			−		
	−		−			−	−	
	−		+			−	+	
	−		+			−	+	
	+		+			−	+	
	+		+			−	+	
			+	+	++	++	+++	
−	−		−		−	−	−	−
			+	−	+	−	+	
	−	−	−		−	−	−	−
	−	−	−		+	−	−	−
	−	−	−		+	−	−	−
	−	+++	+		+		−	−
+	−		++		+	−	+++	+
+++		++	+++	++	++	++	+++	+++
			+	++	+		−	
+++		++	+++	++	++	+++	+	
			++	++	++	++	+++	
			+	−	++	++	+	
			+	+	++	++	+	
−	+++		+		+	+	−	−
−	−		+		−	−	−	−
−	+		++	+	+		++	
	−	−	−	−	−	−	+++	−
	−	−	−	−	−	−	−	−
	−	+	−		−	−	+	−
	−	+	−		−	−	−	−
	−	−	−		−	−	−	−
					+	−	−	−
					+	−	−	−
					+	−	++	+++
+++	++	++	++	+	+	−	+++	+++
+++	+++	+++	++	+++	+	+	−	++
−	−	−	−	−	+	−	−	++
−	−	−	+	+	+	−	−	−
	−	+	−		+	−	+	+++
+++	+	++	++	+	++	−	++	+++
+++	+	++	+++	++	++	+	−	−
++	−		++		−	+	−	+
		++	++	++	+	++		++
++	++		++			−	−	+++
++	++	++	+++	−	++		++	+++
++	+	+++	++	+	+	−	−	+
−	−		++		−	−	−	++
++	++	−	++	+	++	+	++	+++
++	++	+++	++	+	++	−		
			+	++	−	+	−	
			+	++	+	−		

TABLE 1. Continued.

Taxon	Age of material in years	Source[1]		Enzyme system AAT	AcPH	EST
P. polypodioides	2	Ranker et al. 723	rh[2]			
			lf[2]			
P. polypodioides	1	Ranker et al. 761	rh			
			lf			
*P. virginianum**	0.5	Ranker et al. 839		−	+++	+
*Pteridium aquilinum**	1	Ranker et al. 758			+++	
*P. aquilinum**	0.5	Ranker 816		−	+++	+++
Selaginella apoda	70	Seymour 321		−	−	
S. apoda	36	Adams 49-274		−	−	
S. apoda	14	Thomas 22467		−	−	
S. apoda	3	Yatskievych & Beetle 81-347		−	−	
S. arizonica	18	Keil & Lehto 1146		+	−	
S. bigelovii	28	Hartman 1271		+	−	
S. densa	31	Taylor 7394				−
S. densa	23	LLK 173				+
S. densa	17	Stephens & Brooks 23262		++	−	
S. densa	12	Stephens 66679				++
S. densa	4	Rohde & Fulton 2961				++
S. densa	3	Warner 1233		++	−	
S. lepidophylla	19	Wilson 11400				++
S. lepidophylla	2	Villarreal et al. 2270				++
S. pallescens	1	Ranker et al. 774		−	−	−
S. rupestris	53	Thompson s.n.		−	−	
S. rupestris	39	McGregor 966		−	−	
S. rupestris	29	Lathrop 1993		+	−	
S. rupestris	20	Harms 2759		+	−	
S. rupestris	18	Brooks 455		+	−	
S. rupestris	9	Churchill 7643		+	−	
S. rupestris	5	H & S 5332		+	−	
S. siberica	10	Taylor 19764		+	−	
S. tortipila	35	Sargent s.n.				++
S. tortipila	15	Pittillo et al. 3164				++
S. underwoodii	17	Stephens & Brooks 21869				++
S. underwoodii	10	Brooks & Hauser 10737				++
S. underwoodii	1	Brooks 17210				++
*S. underwoodii***	1	Brooks 17185				++
S. wallacei	64	Otis 1073		+	−	
S. weatherbiana	23	LLK 193				++
S. weatherbiana	15	Stephens & Brooks 42983				++
Woodsia ilvensis	0.5	Ranker et al. 832		+++		
*W. mexicana***	0.5	Windham & Czech 591				
*W. mexicana***	0.5	Windham & Haufler 644				
*W. mexicana***	0.5	Windham & Yatskievych 781				
*W. oregana***	0.5	Windham 423				
*W. oregana***	0.5	Windham & Haufler 652				

Explanation of symbols: no entry: enzyme not assayed; −, no enzymatic activity detected; +, activity present, but not resolvable into discrete bands; ++, bands evident but not clearly resolved, probable secondary isozymes or artifacts present; +++, clearly resolved bands.

* Pressed specimen compared to living material (sporophytes or gametophytes) of same species, different individual.

** Pressed specimen compared to living material of same individual.

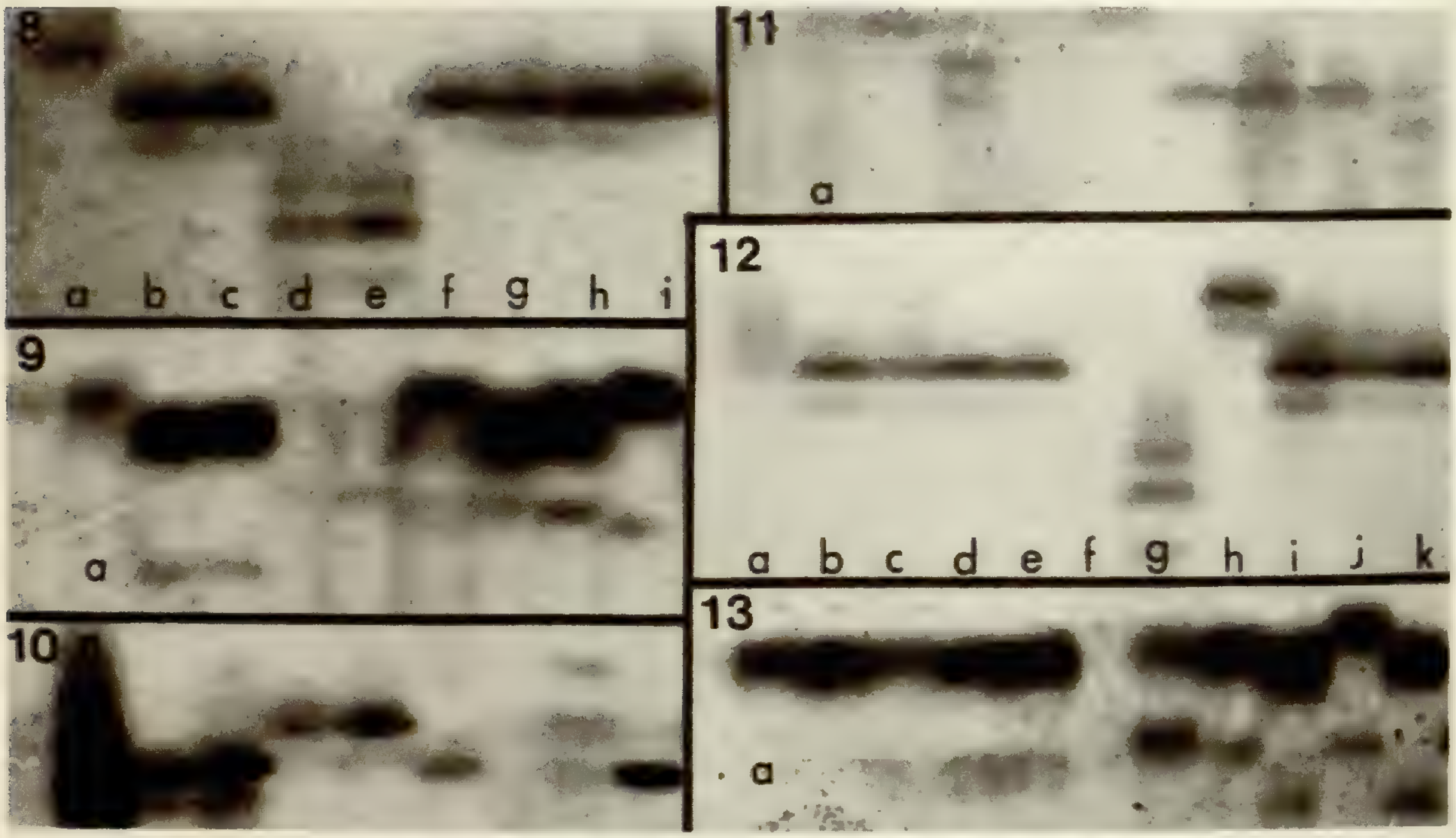
TABLE 1. Extended.

Enzyme system								
HK	IDH	LAP	MDH	6-PGD	PGI	PGM	SkDH	TPI
+++	++	+++	++	++	+++	++	++	+++
+++	++	+++	++	++	++	++	++	+++
+++	++	+++	++	++	+++	++	++	+++
+++	−	+++	−	−	−	−	−	+++
+++	+++		++		++	+++	−	++
			−	−	+	−	+	
	−				+	+	−	−
−	−	−	−		−		−	−
−	−	−	−		−		−	−
−	−	−	−		−		−	−
−	−	−	−		−		−	−
++	++	+++	++		++		+++	+++
−	++	+++	+		++		++	++
	−	+++	+		+	−	−	+
	+	+++	+		+	−	+	+++
++	++	+++	++		++		+++	+++
	++	+++	++		++	−	++	+++
	+	+++	++		++	−	++	+++
+	+	+++	+++		++		+++	+++
	++	++	+		−	−	++	+++
	++	++	++		++	−	++	+++
	++		++	++	++	++	+++	−
−	−	−	−		−		−	−
−	−	−	+		+	−	++	
−	+	++	+		+		−	++
−	−	++	++		++		−	+++
−	++	++	++		++		+++	+++
+	++	+++	+++		++		+++	+++
+	+	+++	++		++		+++	+++
	−	+	+		+		+++	−
	+++	++	++		++	−	+++	+++
	+	+++	+++		++	−	+++	+++
	+	+++	+++		++	−	+++	+++
	++	+++	+++		++	−	++	+++
	++	+++	+++		++	−	+++	+++
−	−	−	+		−		+	−
	+	++	++		+	−	+++	+++
	+	+++	++		++	−	+++	+++
			+	++	++	++	+++	
+++		++	+++	+++	+++	+++	+++	+++
+++		++	+++	+++	+++	+++	+++	+++
++		++	++	+++	+++	+++	+++	+++
+++		++	+++	+++	+++	+++	+++	++
+++	++	+++		+++	+++	+++	+++	+++

*** Specimen originally labeled *S. weatherbiana* but electrophoretically identified as *S. underwoodii*.

[1] Voucher specimens deposited at KANU, unless otherwise noted.

[2] rh = rhizome extract; lf = leaf extract.



FIGS. 8–13. Photographs of gels. Anode is at the top of each figure. Lane a is marked for reference in all figures. The same individuals are represented in Figs. 8–11. Species (age of specimen in years) are: a, b, c: *Selaginella densa* (23, 12, 4, respectively); d, e: *S. weatherbiana* (23, 15, respectively); f, g, h, i: *S. underwoodii* (1, 17, 10, 1, respectively). FIG. 8. TPI. FIG. 9. LAP. FIG. 10. EST. FIG. 11. MDH. Figs. 12 and 13 are of the same individuals. Species (age) are: a–e: *S. rupestris* (29, 20, 18, 9, 5, respectively); f: *S. wallacei* (64); g: *S. bigelovii* (28); h: *S. arizonica* (18); i: *S. densa* (17); j: *S. siberica* (10); k: *S. densa* (3). FIG. 12. TPI. FIG. 13. MDH.

ute to the ability of these taxa to withstand desiccation. While the potential of the specimens we studied to resume *in vivo* physiological function was not assessed, our results show that the extreme desiccation tolerance of these species allows, at least, for the consistent preservation of some enzymes for more than 25 years. The observation that identical isozyme bands were expressed in conspecific individuals sampled at approximately 10-year intervals over 20- to 40-year periods, suggests that no changes in molecular confirmation occurred at most loci. Thus, while comparisons with live material should be done in any study that incorporates dried specimens, this may be less crucial with species of this genus.

The ability to recover well-resolved isozymic patterns from herbarium specimens also provides an opportunity to use electrophoretic markers to check the classification of specimens of questionable identity. This may be useful particularly in the taxonomically difficult genus *Selaginella* due to the cryptic nature of most of the characters used in distinguishing species (Tryon, 1955). An example of this was provided inadvertently in the present study. One of the one-year-old herbarium specimens of *S. underwoodii* (Table 1) actually was labeled *S. weatherbiana*. Electrophoretically, however, it was found to be quite distinct from the other specimens of the latter species and shared most of its allozymes with *S. underwoodii* (Figs. 8–11, lane f). Reinspection of the specimen revealed that, in fact, it had been misidentified. Larger sample sizes are needed to deter-

mine whether the genomic markers are absolute (i.e., mutually exclusive) but these data show that the use of electrophoresis may be of value in identifying morphologically confusing individuals.

Conclusions

There are two situations where the inclusion of dried pressed specimens in electrophoretic investigations appears most promising: 1) when recently collected material is available (i.e., six months or less) and 2) when studying certain xerically adapted taxa. The practical implications of the first situation are many. For example, if it is difficult, not desirable, or impossible to collect live whole plants, leaves may be returned to the laboratory in a plant press for subsequent electrophoresis. The results from *Woodsia* and *Pellaea* suggest that this may be possible for a wide variety of taxa. Certainly, press-drying is not the method of choice in preserving leaves to be used for electrophoretic studies, but it does provide a viable and, perhaps, heretofore unappreciated alternative. If leaves are to be preserved by pressing, the method of drying should be considered since it is likely that enzyme activity and integrity will be preserved better under ambient temperature drying than under drying with heat (see Todd, 1972; Eickmeier, 1979). For example, the leaves of *Woodsia* and *Pellaea* were press-dried under ambient temperatures whereas those of *Bommeria pedata* were dried with the aid of a portable electric plant dryer (compare these taxa in Table 1). While the effects of microwave drying on enzymatic activity were not investigated, Bacci et al. (1985) showed a significant loss of integrity of the internal leaf anatomy in several angiosperm species when so treated. Since such destruction of the cellular environment might disrupt enzyme form and function, a study of the effects of microwave drying on enzymatic activity is of interest.

In the second situation, where the taxa under study are xerophytes, electrophoretic sampling could be conducted over a much broader geographic region than that which could be visited by an investigator in a limited period. The taxonomic scope of an investigation also could be expanded to include taxa not collected for various reasons. Herbarium specimens would be indispensable in this regard (see Windham & Haufler, 1986, for a further discussion). Since such small quantities of material are necessary (i.e., 5–15 mg), the damage to preserved specimens is negligible and, in our view, far outweighed by the information gained. We suggest, however, that removal of material from a borrowed herbarium specimen should be done only with the explicit permission of the appropriate curator.

In taxa that exhibit particularly strong preservative qualities (e.g., *Selaginella*), there is the possibility of adding a temporal element to populational studies. For example, the use of herbarium specimens could provide investigators with the opportunity to evaluate the dynamics of the genetic structure of a taxon in a given region through time. Such data could potentially be used to increase the power of studies on mating systems, genetic drift, founder effects, gene flow, and migration, among others (see Windham & Haufler, 1986).

The results of the present study are preliminary but suggest that pressed spec-

imens of various groups of pteridophytes may be used to expand the taxonomic and geographic scope of biosystematic studies. Other fern biologists are strongly encouraged to explore further this potential in their particular groups of interest.

We are grateful to Ralph Brooks of KANU and to the curators of ASU, COLO, and UNM for permission to remove small quantities of leaf material and spores from pressed specimens. Genie Trapp and Chris Haufler kindly provided helpful comments on the manuscript. We thank Dr. Gerald J. Gastony and an anonymous reviewer for their valuable suggestions, Craig Martin for helping with literature references, and Alan Smith for checking the identity of several specimens.

Literature Cited

Bacci, M., A. Checcucci, G. Checcucci, and M. R. Palandri. 1985. Microwave drying of herbarium specimens. Taxon 34:649–653.

Bergtrom, G., M. Schaller, and W. G. Eickmeier. 1982. Ultrastructural and biochemical bases of resurrection in the drought-tolerant vascular plant, *Selaginella lepidophylla*. J. Ultrastruct. Res. 78:269–282.

Bewley, J. D. 1979. Physiological aspects of desiccation tolerance. Annual Rev. Pl. Physiol. 30:195–238.

——— and J. E. Krochko. 1982. Desiccation-Tolerance. Pp. 325–378 in *Encyclopedia of plant physiology*, New Series Volume 12B, *Physiological plant ecology II*, eds. O. L. Lange et al. Berlin-Heidelberg: Springer-Verlag.

Crowe, J. H. and J. S. Clegg. 1978. *Dry biological systems*. New York: Academic Press.

Eickmeier, W. G. 1979. Photosynthetic recovery in the resurrection plant *Selaginella lepidophylla* after wetting. Oecologia 30:93–106.

———. 1980. Photosynthetic recovery of resurrection spikemosses from different hydration regimes. Oecologia 46:380–385.

———. 1983. Photosynthetic recovery of the resurrection plant *Selaginella lepidophylla* (Hook. and Grev.) Spring: effects of prior desiccation rate and mechanisms of desiccation damage. Oecologia 58:115–120.

Gastony, G. J. and D. C. Darrow. 1983. Chloroplastic and cytosolic isozymes of the homosporous fern *Athyrium filix-femina* L. Amer. J. Bot. 70:1409–1415.

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7:1–46.

Harris, H. and D. A. Hopkinson. 1976. *Handbook of enzyme electrophoresis in human genetics*. Amsterdam: North-Holland Publishing Co.

Haufler, C. H. 1985. Enzyme variability and modes of evolution in the fern genus *Bommeria*. Syst. Bot. 10:92–104.

Iljin, W. S. 1957. Drought resistance in plants and physiological processes. Annual Rev. Pl. Physiol. 8:257–274.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. IV. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. Univ. Texas Publ. 7103:49–90.

Soltis, D. E. 1986. Genetic evidence for diploidy in *Equisetum*. Amer. J. Bot. 73:908–913.

———, C. H. Haufler, and G. J. Gastony. 1980. Detecting enzyme variation in the fern genus *Bommeria*: an analysis of methodology. Syst. Bot. 5:30–38.

———, ———, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern. J. 73:9–27.

Stuart, T. S. 1968. Revival of respiration and photosynthesis in dried leaves of *Polypodium polypodioides*. Planta 83:185–206.

Todd, G. W. 1972. Water deficits and enzymatic activity. Water Deficits and Plant Growth 3:177–216.

Tryon, R. M. Jr. 1955. *Selaginella rupestris* and its allies. Ann. Missouri Bot. Gard. 42:1–99.

UPHOF, J. C. TH. 1920. Physiological anatomy of xerophytic Selaginellas. New Phytol. 19:101–131.

WEBSTER, T. R. and T. A. STEEVES. 1964. Observations on drought resistance in *Selaginella densa* Rydb. Amer. Fern J. 54:189–196.

WERTH, C. R. 1985. Implementing an isozyme laboratory at a field station. Virginia J. Sci. 36:53–76.

WINDHAM, M. D. and C. H. HAUFLER. 1986. Biosystematic uses of fern gametophytes derived from herbarium specimens. Amer. Fern J. 76:114–128.

———, P. G. WOLF, and T. A. RANKER. 1986. Factors affecting prolonged spore viability in herbarium collections of three species of *Pellaea*. Amer. Fern J. 76:141–148.

American Fern Journal 76(3):114–128 (1986)

Biosystematic Uses of Fern Gametophytes Derived from Herbarium Specimens

MICHAEL D. WINDHAM and CHRISTOPHER H. HAUFLER
Department of Botany, University of Kansas, Lawrence, Kansas 66045

Ferns and fern allies differ from other vascular land plants in having a completely independent gametophytic generation. Although relatively small and easily overlooked, fern gametophytes have their own set of morphological features, growth conditions, and habitat preferences. Among the ferns, it is the gametophytes that are responsible for both sexual reproduction and initial habitat selection, and knowledge of their physiological tolerances and breeding system characteristics is indispensible when trying to understand patterns of dispersal migration, and speciation. Thus, although physically dwarfed by the sporophytes, fern gametophytes play a key role in the evolutionary process and any "biosystematic" study should include analyses of the gametophytic generation.

Unfortunately, fern gametophytes are seldomly collected and, even when they are, many of the characteristics that make them biologically important cannot be preserved. Therefore, pteridophyte biosystematists must study living cultures of gametophytes. Such cultures are generally initiated by collecting fresh sporophytes, harvesting their spores, and sowing these spores on soil or agar substrates. This approach has yielded a wealth of data, but it has also reinforced the perception that viable spores can only be obtained from recently collected materials. This, in turn, has discouraged the use of fern gametophytes in biosystematic studies since it is difficult to obtain representative samples of widely distributed species and living plants of many narrow endemics may be unavailable.

Although fern biologists interested primarily in sporophyte morphology and anatomy usually depend upon herbarium specimens to circumvent these sampling problems, it was thought that this option was not available to those working with gametophytes. However, recent studies indicate that the spores from herbarium collections of a variety of fern species remain viable for decades. Johnson (1985) reported that the spores of certain *Marsilea* species were capable of germination after 99–100 years, and Windham et al. (1986) germinated 50-year-old spores of *Pellaea truncata*. Spore longevity of this magnitude may actually be common among ferns, especially in taxa occupying periodically xeric habitats. Pray (in Lloyd & Klekowski, 1970) reported that most cheilanthoid fern spores germinated after 10 years, and spores from a specimen of *Notholaena standleyi* at KANU (*Brooks 489*) remained viable after 19 years in the herbarium (Windham, unpubl. data). Although spores of some mesic species such as *Cystopteris protrusa* and *Woodsia obtusa* lose their ability to germinate after a few years, prolonged spore viability has been reported in several ferns inhabiting moist environments. Spores of the tropical species *Asplenium serra* germinated after 48 years (Fischer, 1911), and those of *Polypodium glycyrrhiza* remain viable for at least five years (T. Ranker, pers. comm.).

Aside from the genetic constitution of the plant, the most important factor controlling spore longevity in herbarim collections appears to be the type of treatment used to reduce insect infestation (Windham et al., 1986). Although spore viability declines rapidly in specimens fumigated with methyl bromide, Dowfume or heat, exposure to PDB or low temperatures (−20°C) seems to have little adverse effect. To date, most surveys of spore longevity have focused on materials from larger herbaria using the more toxic fumigants. Thus, there is reason to believe that prolonged spore viability is more prevalent among pteridophytes than the literature would indicate. This raises the possibility that spores from pressed specimens might be used for large scale analyses of gametophytes, which would improve both the scope and quality of biosystematic investigations in ferns.

The present paper explains how herbarium specimens can be employed to circumvent sampling limitations in biosystematic studies by providing a ready source of gametophytic material. Examples from ongoing research using herbarium-derived gametophytes demonstrate ways in which these plants may improve and expand the data base of evolutionary and phylogenetic investigations. Although there are many possible uses for such gametophytes (Miller, 1968; Dyer, 1979), the present discussion focuses on morphological, chromosomal, and genetic evidence that can be obtained from pressed plants. The techniques described herein have proven especially valuable in *Pellaea* and other closely related genera, but it is apparent that they can be useful even in species where spore viability does not exceed five years.

Materials and Methods

Spores were sown and gametophyte cultures maintained using the methods outlined by Windham et al. (1986). Samples showing low germination percentages (<1%) were excluded from the analyses to minimize the possibility of contamination. Morphological features were routinely studied through a Wild dissecting microscope, though a Zeiss phase contrast microscope was used for detailed observations of gametophytes cleared with a saturated solution of chloral hydrate. For chromosomal investigations, gametophytes with a well-developed meristem (6–12 weeks old) were submerged in a 0.2% colchicine solution for 24 hours prior to being placed in Farmer's fixative (3 parts ethanol : 1 part glacial acetic acid). After 24 hours in the fixative, gametophytes were rinsed in distilled water and hydrolized in full-strength Glusulase (snail cytase) for three hours. Quite fragile at this point, they were then carefully rinsed in two changes of distilled water and stored in fresh fixative at −20°C. Chromosome slides were prepared by excising the meristematic regions of several gametophytes, macerating them in 1% iron acetocarmine, and squashing in a 1:1 mixture of acetocarmine and Hoyer's medium. Cells containing chromosomes were photographed using the equipment and materials described by Haufler et al. (1985).

Enzymes for electrophoretic analyses were extracted by crushing gametophytes in a plastic weigh boat with a glass rod after adding 2–5 drops of the phosphate grinding buffer-polyvinylpyrrolidone solution of Soltis et al. (1983).

Gametophytes were ground individually for progeny analyses of meiotic segregation or crushed in groups of 8–10 to determine the isozyme phenotype of the pressed parental plant. The modified gel-electrode system number 11 described by Haufler (1985a) was used to run isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and shikimate dehydrogenase (SkDH). The enzymes aspartate aminotransferase (ATT), hexokinase (HK), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), and triosephosphate isomerase (TPI) were run using Haufler's (1985a) modification of gel-electrode system number 8. The standard staining recipes of Soltis et al. (1983) were followed for AAT, LAP, MDH, and PGM. Agarose overlay stains (Soltis et al., 1983) were used to assay for IDH, HK, PGI, SkDH, and TPI. Photographs of stained gels were taken with a Pentax 35-mm camera using a red filter and Kodak Technical Pan film.

Morphology

Although classifications and phylogenetic hypotheses in ferns have traditionally focused on attributes of the sporophyte, it is clear that morphological evidence from the gametophytic generation can make a major contribution to our understanding of pteridophyte evolution (Stokey, 1951; Nayar & Kaur, 1971; Atkinson, 1973). However, a much wider sampling of taxa will be necessary to understand evolutionary trends in the haploid generation. Herbarium collections form a logical starting point for surveys of gametophyte morphology since they provide an extensive sample of both geographic and taxonomic diversity. Two examples drawn from studies of cheilanthoid ferns illustrate the potential value of this approach.

During research on the dark-stiped members of *Pellaea* section *Pellaea*, Pray (1968a) germinated spores from 10–15-year-old herbarium specimens in order to augment gametophyte samples derived from freshly collected sporophytes. By this means, he was able to obtain living materials of all species in the group and produce one of the best descriptive studies of cheilanthoid gametophytes available. Investigations of the light-stiped members of section *Pellaea* could benefit similarly from analyses of prothalli derived from spores of herbarium specimens. For example, Pray (1968b) observed unusual growth patterns in a collection of *P. andromedaefolia* from Coronado Island and suggested that insular populations might produce gametophytes that are morphologically distinct from those of mainland populations. Unfortunately, he was unable to obtain recently collected materials from other island localities, and most of California's Channel Islands are now off limits to botanical collecting. However, his hypothesis could still be tested by germinating spores from pressed plants collected when insular populations were more accessible.

Ongoing studies of the genus *Notholaena* provide another indication of the value of herbarium specimens for analyses of gametophyte morphology. As noted by Tryon and Tryon (1982), the prothalli of several species of *Notholaena* exhibit glandular trichomes that produce a farinose exudate similar to that observed on the sporophytes. This character could be of great value in clarifying

evolutionary relationships within this taxonomically contentious group of cheilanthoid ferns. However, a general survey of *Notholaena* based solely upon fresh material is not feasible given the highly restricted distribution of some species (Tryon, 1956). An investigation of this group using a combination of herbarium specimens and live plants seems most appropriate and, to date, more than half of the species in the genus have been examined. Glandular gametophytes appear to be characteristic of all species included in the section *Notholaena* by Tryon and Tryon (1982). On the other hand, farinose trichomes are absent from the prothalli of species in section *Argyrochosma*, providing further evidence that this species cluster represents a separate evolutionary line.

These and other examples (Atkinson, 1973; Nayar & Kaur, 1971) amply demonstrate the value of the independent data set that can be obtained from the study of gametophyte morphology. Aside from the natural populations themselves, herbarium collections provide the most extensive sample of fern diversity available. The successful culture of gametophytes derived from the spores of herbarium specimens obviates the perceived requirement for fresh material and prothalli obtained in this manner can (and should) figure prominently in future surveys of morphological variation.

Chromosomal Studies

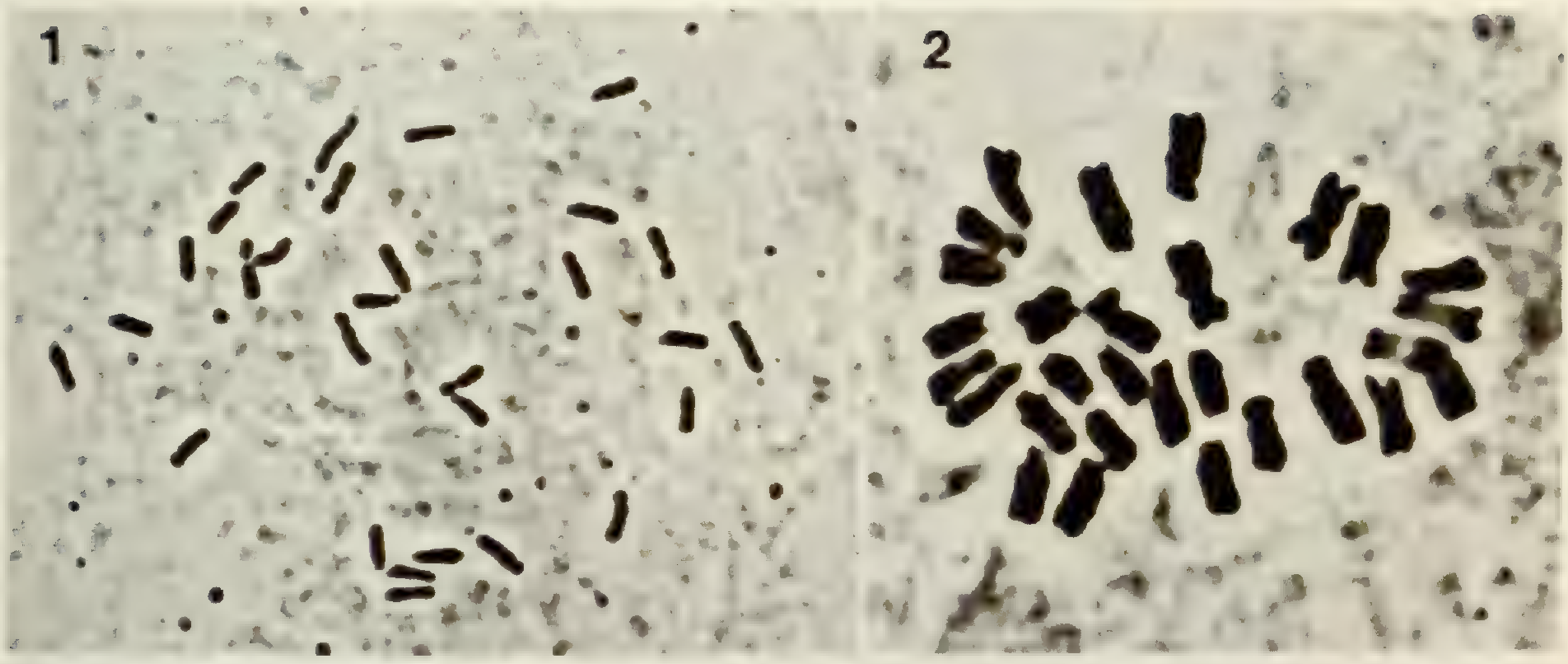
As indicated by Walker (1973), ferns have been the subject of a tremendous amount of cytogenetic research during the past thirty years and, as a result, our understanding of phylogenetic relationships has greatly improved. Many of the species for which chromosomal data are currently lacking occupy relatively inaccessible regions of the world, and gametophytes derived from herbarium collections may be of value in these situations. For example, the first chromosome counts for *Bommeria knoblochii*, a taxon endemic to the rugged mountains of Chihuahua, Mexico, were obtained from the gametophytic progeny of plants collected as herbarium specimens (Gastony & Haufler, 1976). This approach may prove especially valuable in the genus *Notholaena* where endemism is well-developed and few species have been investigated cytogenetically (Tryon & Tryon, 1982).

Although it will be useful to ascertain the chromosome numbers of all fern species, other important goals can be achieved using gametophytes derived from herbarium specimens. Recent cytogenetic studies have revealed an increasing number of cytotypes, cryptic species, and undetected hybrids (e.g., Smith & Foster, 1984; Walker, 1985), and further investigations along these lines are likely to have a significant impact on studies of fern evolution. The assumption that a single chromosome count is characteristic of each morphologically defined taxon has proven false in many cases (e.g., Wagner et al., 1965; Tryon, 1968; Haufler et al., 1985), and more intensive sampling at an intraspecific level is clearly necessary. As with most organisms, fern cytotypes appear to maintain their genetic distinctness through ecological or geographic separation. Thus, herbarium specimens collected throughout the known range of a taxon are especially well-suited to an investigation of intraspecific chromosomal variability.

Since there is a positive correlation between chromosome number and spore size in most ferns (Barrington et al., 1986), cytogenetic work on herbarium specimens with unusual spore measurements may be the most efficient way to detect new cytotypes. It was this approach that led to the discovery of a diploid form of *Woodsia obtusa* (Windham, unpubl. data), a species that Brown (1964) considered an ancient autotetraploid derived from *W. oregana*. This example supports Walker's (1985) assertion that correlations between spore size and ploidy level must be based upon a sufficient number of intraspecific chromosome counts. Brown (1964) did not detect cytotypes during a limited cytogenetic survey of *Woodsia*, but his spore measurements were derived from a large sample of herbarium specimens that was almost certain to include chromosomal variability. In this case, the failure to restrict spore measurements to cytological vouchers led to the exceptional (and erroneous) conclusion that spore length and ploidy level were not correlated in *Woodsia*. Brown's approach to what was then a cytologically unknown group is not unusual, and many cytologists do not measure spores from the same plant that provided the chromosome count. This reflects the fact that sporophytic materials collected for meiotic chromosome studies rarely exhibit mature spores. However, given the amount of intraspecific chromosomal variability detected in recent years, it is clear that spore measurements included in correlative studies must be derived from the actual plant that yielded the count. This relationship is most easily maintained by doing chromosome counts from herbarium specimens where the mature spores themselves provide the gametophytic material used to determine chromosome number.

Although different species often show parallel correlations between spore size and ploidy level, interspecific comparisons must be approached cautiously (Stebbins, 1950). A disjunct population of *Pellaea breweri* from southern Nevada exhibited unusually large spores averaging 54.3 μm in length. However, a chromosome count of $n = 29$ (Fig. 1) based on gametophytes from a 10-year-old herbarium specimen agreed with the only previous report for the species (Tryon & Britton, 1958). In this case, spore size comparisons with closely related species would falsely indicate that the southern Nevada population of *P. breweri* was polyploid, concealing the non-chromosomal nature of spore length variation within this taxon. Since the species in many fern genera are poorly defined and difficult to identify, it might be wise to develop separate correlations for each major geographic region currently included in the distribution of a single species. Herbarium collections, which represent the summation of distributional knowledge, would be an especially valuable resource in this regard. Once correlations between spore size and chromosome number are firmly established in a species complex, it becomes possible to identify cytologically unknown collections (Barrington et al., 1986) and accurately map cytotype distributions using pressed specimens (e.g., Tryon, 1968; Smith, 1975). Basic analyses of this sort provide the foundation for additional studies with far-reaching evolutionary implications.

One of the most neglected aspects of fern cytogenetics is the subject of karyotype analysis. As recently as 1985, fern cytologists were still trying to develop a standardized system of data presentation that would facilitate comparative studies of fern karyotypes (Walker, 1985). However, the potential rewards of this



FIGS. 1, 2. Chromosomal squashes obtained from meristematic cells of gametophytes originating from the spores of herbarium specimens. FIG. 1. *Pellaea breweri* (*Fisher 1786*, UNLV), ×1000. Note that a single haploid chromosome set ($n = 29$) is present although the spores from this collection are unusually large. FIG. 2. *Pellaea truncata* (*Nash, McGill, Hensel, Lehto, and Pinkava 10158*, ASU), ×2000. This preparation suggests that gametophytic material may be valuable in obtaining karyotypic information on ferns. Chromosomes are highly condensed, sister chromatids are evident, and chromosomal morphology is variable.

approach easily overshadow the difficulties posed by terminology, technique, and high chromosome base numbers. For the first time, Walker (1985) has demonstrated concrete differences in chromosome morphology between closely related fern species. Although Walker's analyses were based entirely upon root tip preparations, there are advantages to using gametophytic materials for karyotypes. Prothallial meristematic cells contain half the number of chromosomes found in root tip cells, making comparisons between collections more straightforward. Suitable gametophytes can be derived from herbarium specimens without the fieldwork and greenhouse facilities required to obtain root tips from living sporophytes. Gametophytic preparation techniques are no more difficult or time-consuming than those applied to sporophytic tissue (see Gastony & Haufler, 1976), and many aspects of chromosome morphology can be accentuated by pretreatment with 0.2% colchicine for 24 hours prior to fixation. When prepared in this manner, gametophytes derived from a 13-year-old herbarium specimen of *Pellaea truncata* showed all features essential to the construction of a karyotype (Fig. 2). Chromosomal studies are an integral part of the biosystematic approach, and fern gametophytes derived from herbarium specimens seem well-suited for many of the cytogenetic tasks involved in the study of evolution.

EVOLUTIONARY GENETICS

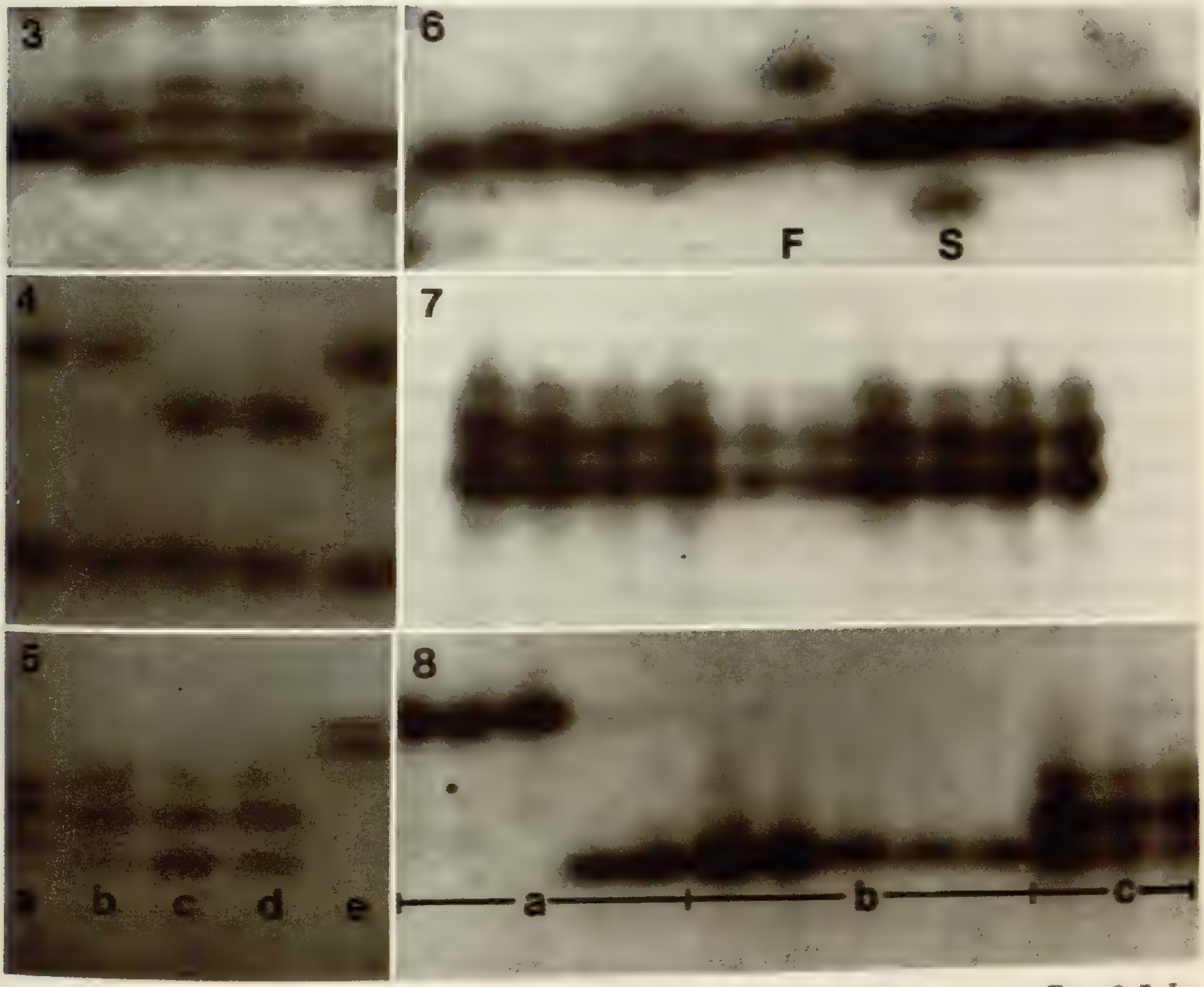
In a recent review, Haufler (1985b) illustrated how electrophoretic analyses of isozymes can make a major contribution to our understanding of pteridophyte evolutionary biology. Although it has been shown that functional enzymes may be extracted from "dead" sporophytic material under certain circumstances (Ranker & Werth, 1986), most electrophoretic work requires the use of living

tissue. For this reason, herbarium specimens have seldom been considered an appropriate source of material for studies of plant isozymes. However, the discovery that some fern spores remain capable of germination for several decades (Windham et al., 1986) suggests that pressed specimens may provide an important data base for future electrophoretic investigations.

Studies of geographic variability.—The power of the electrophoretic technique is directly dependent upon the quality of the available sample. This is especially clear when attempting to characterize a species isozymically. Biosystematic analyses of mixed populations frequently identify potential genomic markers, but these cannot be construed as mutually exclusive until a representative sample of all closely related species has been examined. Growing and studying gametophytes derived from herbarium specimens may be an efficient means to conduct the necessary geographic surveys of enzyme variability.

This approach is currently being used to investigate genetic variability and phylogenetic relationships among three members of the *Pellaea truncata* complex in which long-term spore viability has already been documented (Windham et al., 1986). *Pellaea truncata* (syn. *P. longimucronata*) is a diploid species confined to the southwestern United States and adjacent Mexico (Tryon, 1957). *Pellaea ternifolia* ranges from southern Chile to southern Arizona and includes both diploid and tetraploid cytotypes (Tryon, 1968). Although currently separated by at least 100 km (Windham, unpubl. data), populations of *P. truncata* and diploid *P. ternifolia* apparently hybridized in the past to produce the allotetraploid species *P. wrightiana* (Wagner, 1965). Ongoing electrophoretic studies of these species indicate that freshly collected and herbarium-derived materials provide complementary data sets that can be very useful when investigating isozyme variability. For example, the majority of sporophytes in *P. truncata* are homozygous for a single isozyme at the cathodal locus of PGI (PGI-2). However, electrophoretic analyses of multiple gametophytes from herbarium specimens collected throughout the range of the species occasionally yielded faster (Fig. 6F) and slower (Fig. 6S) bands. Apparently, these isozymes have localized distributions, and they were rare or absent at the localities where living population samples were collected. Some populations of the allotetraploid species *P. wrightiana* show a fixed heterozygotic pattern at the PGI-2 locus which includes a band identical to the faster isozyme seen in *P. truncata* (Figs. 3B, C, D). Since similar bands have not been detected in the *P. ternifolia* parent, it seems likely that this rare *P. truncata* genotype was involved in at least one of the hybridization events that initiated *P. wrightiana*. Thus, a geographic survey of *P. truncata* herbarium specimens has improved our understanding of evolutionary processes in the entire species complex.

Once isozyme markers have been established on the basis of extensive electrophoretic sampling, they can be used to identify specimens in which critical morphological features are atypical, easily misinterpreted, or simply lacking. This can lead to the recognition of cryptic species and interspecific hybrids, both of which provide valuable information concerning phylogenetic relationships. Even supposedly "sterile" interspecific crosses can be studied in this manner because most hybrid ferns produce a small percentage of spores capable of



FIGS. 3–8. Zymograms of gametophytes grown from spores of herbarium specimens. FIGS. 3–5. Individual gametophytes from different sporophytes of *Pellaea wrightiana*. 3 = PGI, 4 = SkDH, 5 = IDH. a = *Windham & Haufler 639*, KANU. b–e = *Windham & Haufler 629*, KANU. The variant, fixed heterozygote electrophoretic phenotypes suggest that sporophytes a, b, c, and e originated independently. Sporophyte d cannot be distinguished from c. FIG. 6. PGI. Spores were obtained from herbarium specimens of *Pellaea truncata* collected throughout the range of the species. Groups of gametophytes were ground together to sample the allelic diversity of the parental sporophyte. Although most sporophytes were monomorphic, a few had rare, variant alleles (labeled F and S). Note that although PGI is a dimeric enzyme, no heterodimeric bands are formed because the allozymes were produced by separate gametophytes. FIG. 7. IDH. Gametophytes from viable spores obtained from a "sterile," triploid, interspecific hybrid between *Pellaea truncata* and *P. wrightiana* (*Windham & Windham 434*, ASC). Note that the electromorphs are monomorphic and that the doses of the component alleles of this fixed-heterozygote are not equal. FIG. 8. IDH. A set of individual gametophytes surveyed to determine the genotypic constitution of sporophytes. a = a heterozygotic diploid of *Pellaea truncata* (*Cottam 14452*, HUH) showing segregation of the two alleles. b = a homozygote of *P. truncata* (*Craig s.n.*, UNM). c = fixed heterozygosity among the gametophytes of the allotetraploid *P. wrightiana* (*Clark s.n.*, UNM). These plants are also notable for the remarkable longevity of their spores. "a" was collected in 1956, "b" in 1936, and "c" in 1944.

germination (DeBenedictus, 1969). Individuals with an unbalanced combination of genomes (such as allopolyploid backcrosses) can often be identified solely by differences in the relative dosages of isozymes (Haufler et al., 1985; Gastony, 1986).

Among temperate species of *Pellaea*, hybridization appears to be most com-

mon between the diploid *P. truncata* and the tetraploid *P. wrightiana* (Windham, unpubl. data). Pray (1971) reported signficant spore germination in one of these hybrids and, during the present study, spores were sown from several herbarium specimens that were morphologically intermediate between *P. truncata* and *P. wrightiana*. Although most of the spores from these plants were malformed and failed to germinate, the larger "basketball" spores did produce prothalli. Electrophoretic progeny analyses using individual gametophytes from one of these collections revealed a strong dosage effect in IDH (Fig. 7). The cathodal and heterodimeric bands were much darker than the anodal band, suggesting that two of the three genomes in the parental sporophyte coded for the cathodal isozyme. This cathodal band is found in both *P. truncata* and *P. wrightiana*, though in the latter species it is always combined with another isozyme to form a fixed heterozygotic pattern (Fig. 8C). The plant in question probably arose through cross fertilization between gametophytes with the IDH genotypes shown in Figures 8B and 8C. Thus, electrophoretic analyses of gametophytes derived from this herbarium specimen support its identification as a triploid hybrid between *P. truncata* and *P. wrightiana*.

At least 43% of homosporous ferns are polyploid (Vida, 1976), and electrophoretic analyses can provide a great deal of information concerning the origin and subsequent evolution of these species. Polyploid taxa normally exhibit fixed heterozygotic banding patterns for a number of enzyme loci (Werth et al., 1985a) and, in most cases, the gametophytes obtained from them have the same complex patterns as the parental sporophyte and are genetically identical to one another. Thus, electrophoretic surveys of enzymes can be used to estimate the ploidy level of gametophytes from herbarium specimens without recourse to chromosomal techniques. For example, progeny arrays from heterozygous sporophytes of the diploid species *Pellaea truncata* show segregation of isozyme bands in IDH (Fig. 8A). Gametophytes from the allotetraploid *P. wrightiana*, on the other hand, exhibit fixed heterozygotic banding patterns because the prothalli themselves are diploid and each contains two divergent genomes (Fig. 8C). The observation of fixed heterozygosity in the gametophytic progeny of a hybrid between *P. truncata* and *P. wrightiana* (Fig. 7) also identifies this plant as a polyploid. The absence of isozymic variability among these gametophytes suggests that most viable spores are the result of non-reductive sporogenesis.

Once a polyploid is identified, isozyme evidence can help to determine whether the taxon originated through interspecific hybridization (allopolyploidy) or intraspecific chromosome doubling (autopolyploidy). An allopolyploid should contain genomic markers from each species involved in its hybrid origin (Werth et al., 1985a). The detection of a polyploid containing the markers of a single species provides circumstantial evidence that the plant or population arose through autopolyploidy (Haufler et al., 1985). In addition, analyses of gametophytes can be used to distingiush disomic from tetrasomic inheritance and thereby identify potential autopolyploids. Since autopolyploids are often morphologically indistinguishable from their diploid ancestors, electrophoretic analyses of herbarium collections may facilitate their recognition and thus allow biosystematists to assess the evolutionary importance of autopolyploidy in the ferns.

Recent studies involving the Appalachian *Asplenium* complex have demonstrated remarkable congruity between enzyme data and morphologically based hypotheses of relationship (Werth et al., 1985a, 1985b). Each diploid species contained a unique set of isozymic markers, and most hybrid individuals exhibited anticipated patterns of electrophoretic additivity. However, these studies also revealed unexpected evidence of "orphan" alleles, selective gene silencing, and multiple origins among the allopolyploid members of the complex. "Orphan" alleles are those isozymes found in an allopolyploid species which have not been identified in any of the putative diploid progenitors. On the other hand, the absence of one or more ancestral markers from a known allopolyploid is often viewed as evidence of gene silencing. Orphan alleles and apparent gene silencing could both result from incomplete sampling of enzyme variability in the diploid progenitors. The former may represent rare isozymes whose distribution was restricted to a few diploid plants at the locality where the original allopolyploid was formed. The faster migrating PGI-2 isozyme found in some plants of *Pellaea wrightiana* (Figs. 3B, C, D) might have been classified as an orphan allele if its presence at low frequency in *P. truncata* had not been revealed by an electrophoretic survey of gametophytes from herbarium specimens (Fig. 6F). "Silenced" genes may be interpreted as extremely common isozymes which are not found in some members of the hybridizing diploid population and, therefore, cannot be considered absolute genomic markers. It is true that the putative orphan and silenced isozymes might result from mutation, but causal investigations should not be undertaken until the possibility of sampling error is ruled out.

Although the true nature of orphan alleles and silenced genes has not been resolved in ferns, there can be little doubt that many allopolyploids are the result of several, independent hybridization events (Werth et al., 1985b; Haufler, 1985b). Most of the allopolyploid species examined to date show two or more fixed isozyme patterns which represent the amalgamation of genes from different parental populations. Gametophytes from five plants representing two different populations of the allotetraploid *Pellaea wrightiana* serve to illustrate the phenomenon of multiple polyploid origins. In this small sample, there are two PGI-2 genotypes (Fig. 3), two SkDH genotypes (Fig. 4), and three IDH genotypes (Fig. 5). Considering all loci, plants c and d are identical to one another, but each of the other individuals shows a unique genotype. Thus, if there has been no genetic exchange among genotypes in *P. wrightiana* since their origin, the species must have arisen through at least four different hybridization events. Even if genetic exchange and mutation have occurred, at least two separate origins would be required to explain the genetic variability observed in *P. wrightiana*.

Investigations of multiple origins in allopolyploid ferns may shed new light on the biogeographical aspects of speciation and migration. Werth et al. (1985b) stated that "areas now occupied by single genotypes may circumscribe centers of allopolyploid origin." Under this hypothesis, allopolyploid populations showing a mixture of isozyme phenotypes would result from range expansion and secondary contact. However, it is equally plausible that regions showing a diversity of polyploid phenotypes represent the point of origin, and that genetically

uniform, outlying populations result from subsequent migration of single genotypes. The relative merits of these opposing hypotheses cannot be judged solely on the basis of data from the allopolyploid, but the question could be resolved through a careful examination of the diploid progenitors. If rare allopolyploid isozymes can be traced back to their diploid source through electrophoretic surveys of herbarium specimens, it may be possible to determine the approximate point of origin for each enzymatic phenotype observed in the polyploid taxon. This type of research might provide critical information concerning the early stages of allopolyploid speciation and establishment.

Breeding system analyses and crossing programs.—Large-scale geographic surveys of enzyme variability can also improve our understanding of evolutionary mechansims in diploid homosporous ferns. Breeding systems have a profound effect on gene flow, dispersability, and other factors involved in the speciation process. Although Klekowski and Baker (1966) hypothesized that homosporous ferns are predominantly inbreeding, recent studies of populational variability in *Bommeria* (Haufler & Soltis, 1984), *Cystopteris* (Haufler, 1985b), and *Pellaea* (Gastony & Gottlieb, 1985) challenge this interpretation. The breeding system of most pteridophyte taxa remains to be established, and gametophytes derived from herbarium specimens can be useful in several ways. Since the inbreeding potential of some fern species varies from population to population (Cousens, 1979), any attempt to characterize the breeding system should incorporate data from a variety of habitats and localities. If an adequate sample taken throughout the range of a genetically variable diploid species reveals little genetic differentiation between populations, this provides circumstantial evidence that the species is largely outcrossing (Hamrick et al., 1979). If, on the other hand, genetic variants are geographically localized, it is likely that the species is primarily inbreeding. In the latter situation, it may be possible to investigate the rates and patterns of gene flow accompanying divergence between populations because herbarium collections provide a sample of both geographic and temporal variability.

Artificial crossing experiments were considered an integral part of "biosystematy" by those who originally defined the term (Camp & Gilly, 1943), and most systematic studies of seed plants devote a great deal of time and effort to investigations of crossability. Unfortunately, the same cannot be said for ferns, in which the gametophytes necessary for crossing experiments mature slowly and require special growth conditions to insure sporophyte production (Cousens, 1979). Most artificial crosses reported in the fern literature involve interspecific hybridization, a technique routinely used by European pteridologists (Lovis, 1968) but rarely applied in other regions of the world. Crossability has also been used as a measure of intraspecific genetic divergence in *Bommeria hispida* (Haufler, 1979) and *Blechnum spicant* (Cousens, 1979). Information gained from intra- and interspecific crossing experiments can be especially critical in groups such as the ferns where cryptic species are common and the number of phylogenetically useful morphological characters is limited.

Interspecific crosses between genetically different parental populations yield hybrids with distinctive isozymic (Werth et al., 1985b), chromosomal (Wagner,

1971), and morphological (Barrington, 1985) phenotypes. For this reason, evolutionary hypotheses should not be based solely upon the results of a single hybridization experiment or event. Without a doubt, the greatest diversity of hybrids can be obtained by using herbarium collections as the primary source of gametophytes. Lovis (1968) reported that certain natural hybrids, such as *Asplenium* ×*alternifolium*, are extremely difficult to synthesize in the laboratory. This could result from genetic incompatibility between diploid strains used in the hybridization experiments. Thus, attempts to "recreate" a natural allopolyploid might be more successful and informative if the selected diploids were genetically similar to the supposed parents involved in the original cross. Again, electrophoretic surveys of gametophytes derived from a diverse array of pressed plants may help to locate putative parental populations. This approach can also enhance the utility of intraspecific crossing experiments, which have traditionally used morphological differences or geographic isolation as measures of genetic distance. Although morphology and geography often reflect the underlying genetic structure of a species, genetic distances can be calculated more precisely through surveys of enzyme variability. Following this, phenotypic, genotypic, and distributional data can be used to design a crossing program yielding the maximum amount of information on intraspecific genetic diversity.

The temporal component.—Considering all of the potential uses of herbarium specimens in biosystematic research, perhaps the most intriguing involves analyses of genetic change through time. In natural populations that are not subject to strong artificial selection, evolutionary changes are rarely observed during the tenure of most studies (or botanists). Instead, past evolutionary events are inferred from the differential distribution of morphological or genetic traits among modern populations. This approach may be a valid way to detect large-scale evolutionary changes, but it reveals little about the mechanisms involved. Since the evolutionary process is often equated with changes in allele frequencies within a population (Mayr, 1982), mechanistic investigations are probably best initiated at this level.

Through the use of electrophoretic analysis of isozymes, gametophytes from herbarium specimens can generate valuable data concerning genetic change within a population. The only prerequisite for such studies is a representative sample of living plants on which to base estimates of allele frequencies for the modern population. Once this requirement is met, valid comparisons can be made to either isolated plants or population samples previously collected at the same geographic locality. If statistical evaluations of changes in gene frequency are to be undertaken, gametophytes must be available from two or more plants collected at approximately the same time. Thus, the chosen locality must be popular with collectors or visited by a botanist who collected a large number of duplicates. In either case the collections must have been deposited in herbaria where spores are likely to remain viable for long periods (e.g., COLO or UNM; see Windham et al., 1986). This is a highly improbable chain of events, and most analyses of genetic change using fern herbarium specimens will probably take the form of simple probability statements comparing isolated individuals from the past with the modern population. The strongest case for genetic change

would result from the detection of an allele in the pressed plant which was not found in the modern population. Although this is an unlikely situation, powerful probability statements can be generated for any genetic locus (or combination of loci) showing strongly skewed allele frequencies. For example, if a plant collected 50 years ago is homozygous for an allele which has a frequency of 5% in the modern population, the probability is very low (0.0025) that this individual was randomly selected from a genetically identical population. Since the herbarium specimen and modern sample belong to the same spatial population (i.e., they were collected at the same locality), it is likely that they represent different temporal populations. In other words, the probability is high that allele frequencies at this genetic locus have changed during the last 50 years as the population has evolved.

A temporal series of isolated specimens from a single locality could be especially informative when analyzed in conjunction with environmental data (such as those recorded in tree rings at the collection site). If such a series suggested a major change in allele frequencies during a certain interval, it might be possible to correlate this change with an evolutionary bottleneck caused by drought, brush fire, or some other local catastrophe. Causal investigations of this sort have rarely been undertaken in wild populations, but they may be important to our understanding of evolutionary processes. There are potential limitations to this approach, most of which are related to the time span available for study. *Pellaea* and *Marsilea* show continued spore viability after 50 and 100 years respectively (Windham et al., 1986; Johnson, 1985), but substantial changes in gene frequency are not likely to be observed within this time frame without strong selection or some major perturbation of the environment. The probability of detecting such changes will be greatest in short-lived diploid species occupying an unstable habitat, and initial studies should probably focus on populations with these characteristics.

Conclusions

The fern life cycle comprises two completely independent generations, both of which must be investigated if we are to understand the important aspects of pteridophyte biology. Unfortunately, practical limitations have reduced the impact that analyses of the gametophytic generation have made on evolutionary studies. Difficulty in obtaining representative samples of living gametophytes has been the major problem, but this may be overcome in some ferns by germinating spores from herbarium specimens. Studying the gametophytes derived from these spores could improve our sampling of morphological variability and provide a new set of characters for use in phylogenetic analyses. Cytogenetic studies of such gametophytes can be used to document intra- and interspecific differences in chromosome number and may be the most reasonable approach to karyotypic research. Electrophoresis of herbarium-derived gametophytes can expand our otherwise limited sample of isozymic variability, can supply information on the origins of polyploid taxa, and can be used to search for "orphan" alleles. Through crossing programs, gametophytes originating from a diverse array of herbarium

specimens can be useful in documenting genetic discontinuities and recognizing cryptic species. They can also provide a window to the past for analyzing changes in gene frequency over time. Preliminary studies indicate that the usefulness of herbarium collections is not limited to investigations of sporophytic morphology, ecology, and biogeography. Through studies of gametophytes derived from spores, herbarium specimens of ferns can provide a wealth of information on pteridophyte biology and evolution. At present, the only serious barrier to studies of this sort is our lack of knowledge concerning prolonged spore viability. It is hoped that the ideas presented herein will stimulate additional research on spore longevity in a variety of pteridophyte genera. Only then will we be able to assess fully the value of herbarium specimens for biosystematic research on ferns.

Literature Cited

Atkinson, L. 1973. The gametophyte and family relationships. Pp. 73–90 in *The phylogeny and classification of the ferns*, ed. A. Jermy et al., J. Linn. Soc., Bot. 67 (Suppl. 1).

Barrington, D. 1985. The morphology and origin of a new *Polystichum* hybrid from Costa Rica. Syst. Bot. 10:199–204.

———, C. Paris, and T. Ranker. 1986. Systematic inferences from spore and stomate size in the ferns. Amer. Fern J. 76:149–159.

Brown, D. 1964. A monograph of the fern genus *Woodsia*. Nova Hedwigia 16:1–154.

Camp, H. and C. Gilly. 1943. The structure and origin of species. Brittonia 4:323–385.

Cousens, M. 1979. Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*. Amer. J. Bot. 66:116–132.

DeBenedictus, V. 1969. Apomixis in ferns with special reference to sterile hybrids. Unpublished PhD. dissertation, University of Michigan.

Dyer, A. 1979. The culture of gametophyes for experimental investigation. Pp. 253–305 in *The experimental biology of ferns*, ed. A. Dyer. London: Academic Press.

Fischer, H. 1911. Licht- und Dunkelkeimung bei Farnsporen. Beih. Bot. Centralbl. 27:60.

Gastony, G. 1986. Electrophoretic evidence for the origin of fern species by unreduced spores. Amer. J. Bot. 73:1563–1569.

——— and L. Gottlieb. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–267.

——— and C. Haufler. 1976. Chromosome numbers and apomixis in the fern genus *Bommeria*. Biotropica 8:1–11.

Hamrick, J., Y. Linhart, and J. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10:173–200.

Haufler, C. 1979. A biosystematic revision of *Bommeria*. J. Arnold Arbor. 60:445–476.

———. 1985a. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

———. 1985b. Pteridophyte evolutionary biology: the electrophoretic approach. Proc. Roy. Soc. Edinburgh 86B:315–323.

——— and D. Soltis. 1984. Obligate outcrossing in a homosporous fern: Field confirmation of a laboratory prediction. Amer. J. Bot. 71:878–881.

———, M. Windham, D. Britton, and S. Robinson. 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. Canad. J. Bot. 63:1855–1863.

Johnson, D. 1985. New records for longevity for *Marsilea* sporocarps. Amer. Fern J. 75:30–31.

Klekowski, E. and H. Baker. 1966. Evolutionary significance of polyploidy in the Pteridophyta. Science 153:305–307.

Lloyd, R. and E. Klekowski. 1970. Spore germination and viability in Pteridophyta: Evolutionary significance of chlorophyllous spores. Biotropica 2:129–137.

LOVIS, J. 1968. Fern hybridists and fern hybridising. II. Fern hybridising at the University of Leeds. Brit. Fern Gaz. 10:13–20.

MAYR, E. 1982. *The growth of biological thought*. Cambridge: Harvard University Press.

MILLER, J. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–440.

NAYAR, B. and S. KAUR. 1971. Gametophytes of homosporous ferns. Bot. Rev. (Lancaster) 37:295–396.

PRAY, T. 1968a. The gametophytes of *Pellaea* section *Pellaea*: Dark-stiped series. Phytomorphology 18:113–142.

———. 1968b. Interpopulational variation in the gametophytes of *Pellaea andromedaefolia*. Amer. J. Bot. 55:951–960.

———. 1971. The gametophytes of natural hybrids in the fern genus *Pellaea*. Amer. Fern J. 61: 128–136.

RANKER, T. and C. WERTH. 1986. Active enzymes from herbarium specimens: Electrophoresis as an afterthought. Amer. Fern J. 76:102–113.

SMITH, A. 1975. The California species of *Aspidotis*. Madroño 23:15–24.

——— and M. FOSTER. 1984. Chromosome numbers and ecological observations of ferns from El Tirol, Paraguay. Fern Gaz. 12:321–329.

SOLTIS, D., C. HAUFLER, D. DARROW, and G. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

STEBBINS, G. 1950. *Variation and evolution in plants*. New York: Columbia University Press.

STOKEY, A. 1951. The contribution by the gametophyte to classification of the homosporous ferns. Phytomorphology 1:39–58.

TRYON, A. 1957. A revision of the fern genus *Pellaea* section *Pellaea*. Ann. Missouri Bot. Gard. 44: 125–193.

———. 1968. Comparisons of sexual and apogamous races in the fern genus *Pellaea*. Rhodora 70:1–24.

——— and D. BRITTON. 1958. Cytotaxonomic studies on the fern genus *Pellaea*. Evolution 12: 137–145.

TRYON, R. 1956. A revision of the American species of *Notholaena*. Contr. Gray Herb. 179:1–106.

——— and A. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.

VIDA, G. 1976. The role of polyploidy in evolution. Pp. 267–294 in *Evolutionary biology*, ed. V. Novak and B. Pacltova. Praha: Czech. Acad. Sci.

WAGNER, W. Jr. 1965. *Pellaea wrightiana* in North Carolina and the question of its origin. J. Elisha Mitchell Sci. Soc. 81:95–103.

———. 1971. Evolution of *Dryopteris* in relation to the Appalachians. In *The distributional history of the biota of the southern Appalachians. Part 2: Flora*, ed. P. Holt. Va. Polytech. and State Univ., Res. Div. Mono. 2:147–192.

———, D. FARRAR, and K. CHEN. 1965. A new sexual form of *Pellaea glabella* var. *glabella* from Missouri. Amer. Fern J. 55:171–178.

WALKER, T. 1973. Evidence from cytology in the classification of ferns. Pp. 91–110 in *The phylogeny and classification of the ferns*, ed. A. Jermy et al., J. Linn. Soc., Bot. 67 (Suppl. 1).

———. 1985. Cytotaxonomic studies of the ferns of Trinidad 2. The cytology and taxonomic implications. Bull. Brit. Mus. (Nat. Hist.), Bot. 13:149–249.

WERTH, C., S. GUTTMAN, and W. ESHBAUGH. 1985a. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Syst. Bot. 10:184–192.

———, ———, and ———. 1985b. Recurring origins of allopolyploid species in *Asplenium*. Science 228:731–733.

WINDHAM, M., P. WOLF, AND T. RANKER. 1986. Factors affecting prolonged spore viability in herbarium specimens of three species of *Pellaea*. Amer. Fern J. 76:141–148.

American Fern Journal 76(3):129–140 (1986)

Detecting Abortive Spores in Herbarium Specimens of Sterile Hybrids

W. H. WAGNER JR. and F. S. WAGNER
Department of Botany and Herbarium, University of Michigan, Ann Arbor, Michigan 48109
W. CARL TAYLOR
Botany Section, Milwaukee Public Museum, Milwaukee, Wisconsin 53233

Uses of herbarium specimens for biosystematic research are being extended by new techniques, and those described here have been exploited only in the past few decades. Not only do dried and pressed pteridophyte specimens provide such classical gross data as geographical, ecological, and morphological, but microscopic and chemical data as well. Specimens on herbarium sheets often yield hypotheses of hybridity by morphology alone, based on the phenomenon of intermediacy (W. Wagner, 1983), in which the characters of higher plant hybrids tend to have expressions lying between those of the parental species: If one has ovate leaves, and the other lanceolate leaves, the hybrid has more or less ovate-lanceolate leaves. The hybrid's characters are not necessarily in the middle; some may be closer to one parent, some closer to the other.

Probably most investigations of hybrid pteridophytes begin in the herbarium, where specimens showing interspecific intermediacy are encountered. We call such specimens "intertaxa" because they do not fit either presumed parent. Whenever they are found we examine their spores. Interspecific pteridophyte hybrids fall into three categories as regards spore production, namely (a) sterile hybrids that have defective spores (e.g., Fig. 1), (b) fertile hybrids (either introgressant or, much more commonly, allopolyploid) that have normal viable spores, and (c) apogamous hybrids that produce unreduced spores. We are not concerned here with fertile hybrids, except for those that are apogamous (e.g., in the United States, *Pteris* × *hillebrandii* and *Asplenium* × *heteroresiliens*). Obligately apogamous hybrids can usually be recognized because some or most of the sporangia have only 32 spores that are viable; those with 64 spores show abortion (various combinations are also possible; cf. Manton, 1950). Apogamous intertaxa involve one apogamous and one sexual parent. Non-hybrid taxa with normally 32 or 16 spores per sporangium might be confused with apogamous hybrids but these can be recognized because the reduced spore number is regularized, characteristic of all sporangia, and the spores are non-abortive. Examples of 32- or 16-spored sporangia in normal sexual species are found in a wide diversity of genera (e.g., *Ceratopteris, Cheilanthes, Lindsaea, Alsophila, Sadleria*). Allopolyploid sexual hybrids behave like ordinary species, even though they are actually hybrids, and many of these are frequent or common; they are recognized by their spore size, usually larger than that of their parents.

Not all spore abortion is associated with hybridity (see below under "Additional Perspectives"). Nearly all spore samples from normal species show at least some deviant spores. It is not unusual to find as much as 10–20 percent of spores

of normal species to be abortive. The causes for this are not known: it may be genetically determined or produced by environmental conditions. A most interesting group of non-intertaxa with abortive spores is that involving crosses between different ploidal levels of the same species. These are often very hard to recognize on the basis of gross morphological characters. Fortunately plants of this type appear to be uncommon in nature, but it may simply be that they are undetected.

Sterile intertaxa with defective spores are much more common than any other types of hybrids, and it is with these that we are mainly concerned here. When we consider the high incidence of sterile nothospecies among pteridophytes, the use of spore abortion as a means of identification can become extremely important, especially where the parental species are subtly differentiated on the basis of gross morphology. In the scouring rushes, for example, Hauke (1963) showed that several of the species combinations (*E.* ×*trachyodon* [*E. hyemale* × *variegatum*], *E.* ×*ferrissii* [*E. hyemale* × *laevigatum*], and *E.* ×*nelsonii* [*E. laevigatum* × *variegatum*]) are surprisingly common in herbaria. However, they are distinguished by subtle vegetative characters, so that abortive spores provide one of the best aids for their identification.

There are different kinds and degrees of hybrid spore abortion, depending upon the case, and so far as we know, no one has brought these together and classified them. The most complete study of hybrid abortion in ferns is by DeBenedictus (1969). Involved are the relative development of sporangia, deposits of materials on the inner capsular wall, sizes of spores (both the outer and inner diameter of the perispore), spore surfaces, relative wall development (especially the perispore), forms of the laesurae, spore pigmentation, extent of wall collapse, presence and condition of the protoplasts, and germination. Various combinations of these different abortive expressions produce a diversity of syndromes. Indeed, we believe that future studies may show that the nature of spore abortion is just as characteristic of a given hybrid combination as details of its morphology, cytology, or chemistry.

Techniques

The goal is to remove spores without damaging the herbarium specimen and to prepare the spores so that they may be observed and studied readily. The techniques described here apply to leptosporangiate ferns, but they may be readily adapted to other pteridophytes. One must beware of occasional contamination from other specimens; an interesting sterile hybrid may be contaminated with good spores from adjacent sheets or specimens collected at the same time. Also, specimens with partly immature spores can be confusing; the mixture from a half-developed sorus of very young with nearly mature or mature spores may resemble true abortion. Given an uncontaminated and mature herbarium specimen that we want to test for presence of abortive spores, the procedure is as follows:

1. Under dissecting microscope, examine specimen for areas of leaves or stems where there are numerous sporangia that are mature and well-developed. Find

sporangia that have not opened or that have opened only partially. Old, wide-open sporangia may have but few spores remaining, and young, tightly closed sporangia may have only incompletely formed spores, although these can be useful sometimes when abortion shows up early in development.

2. Place drop of Hoyer's Solution (Anderson, 1954) on the middle of a slide and dip the point of a dissecting needle into the solution to wet the tip in order to provide a "glue" for picking up spores.

3. Gather material on tip of needle. For leptosporangiate ferns simply amass a number of spore-filled sporangia on the wet needle-tip. For eusporangiate ferns and other pteridophytes, dig into one or more sporangia so that the needle-tip becomes covered with spores.

4. Transfer spores or sporangia to the drop of Hoyer's Solution on the microscope slide and stir in order to spread the spores evenly. Repeat (3) and (4) as necessary to accumulate roughly a hundred or more spores in the drop.

5. Pass needle-tip through flame of alcohol lamp and burn off all of the remaining spores, sporangial debris, and Hoyer's Solution. Clean the burned tip with paper tissues to prevent contamination when using the same needle on another herbarium collection.

6. Cover spores in the drop of Hoyer's Solution by slowly lowering the cover glass over them. Tap gently on the top of the cover glass with the needle to spread the spores around uniformly and to flatten any sporangial or other soral debris that may be holding up the cover glass. Other media, e.g., diaphane, silica gel, glycerin jelly, may be used for mounting the spores, but in publication this should be stated, so that repeated measurements will be comparable.

7. Under the compound microscope, survey slide looking for evidence of abortion, as described below. If in doubt, make as many additional slides as necessary, following steps (2) through (7).

8. If slide proves to be interesting (as in Fig. 1) and of sufficient significance to retain, allow it to dry along the edges of the cover glass, after removing excess Hoyer's Solution. When the edges become sufficiently dry (after a couple of weeks), ring the cover glass with Glyptal (General Electric Co. product), and permit the sealant to dry. Most herbaria prefer to have good, well-labeled spore slides kept together with the specimen from which the sample came. The completely dried slide may be placed in a pocket directly on the herbarium sheet with the specimen. (Such slides can be broken only by rough handling, not likely to be encountered in a well-kept herbarium.)

Signs of Spore Abortion

Abortion can be characterized as significant variation from the normal products of sporangio- and sporogenesis. The different manifestations in spore abortion are interrelated, probably because they are traceable to the same morphogenetic upsets. For example, spore samples showing exaggerated variability in spore size (Fig. 2B) will show all or most of the symptoms given below. Spore collapse may go hand in hand with loss of the protoplast. Unusually massive perisporial development often occurs together with reduction of the size of the

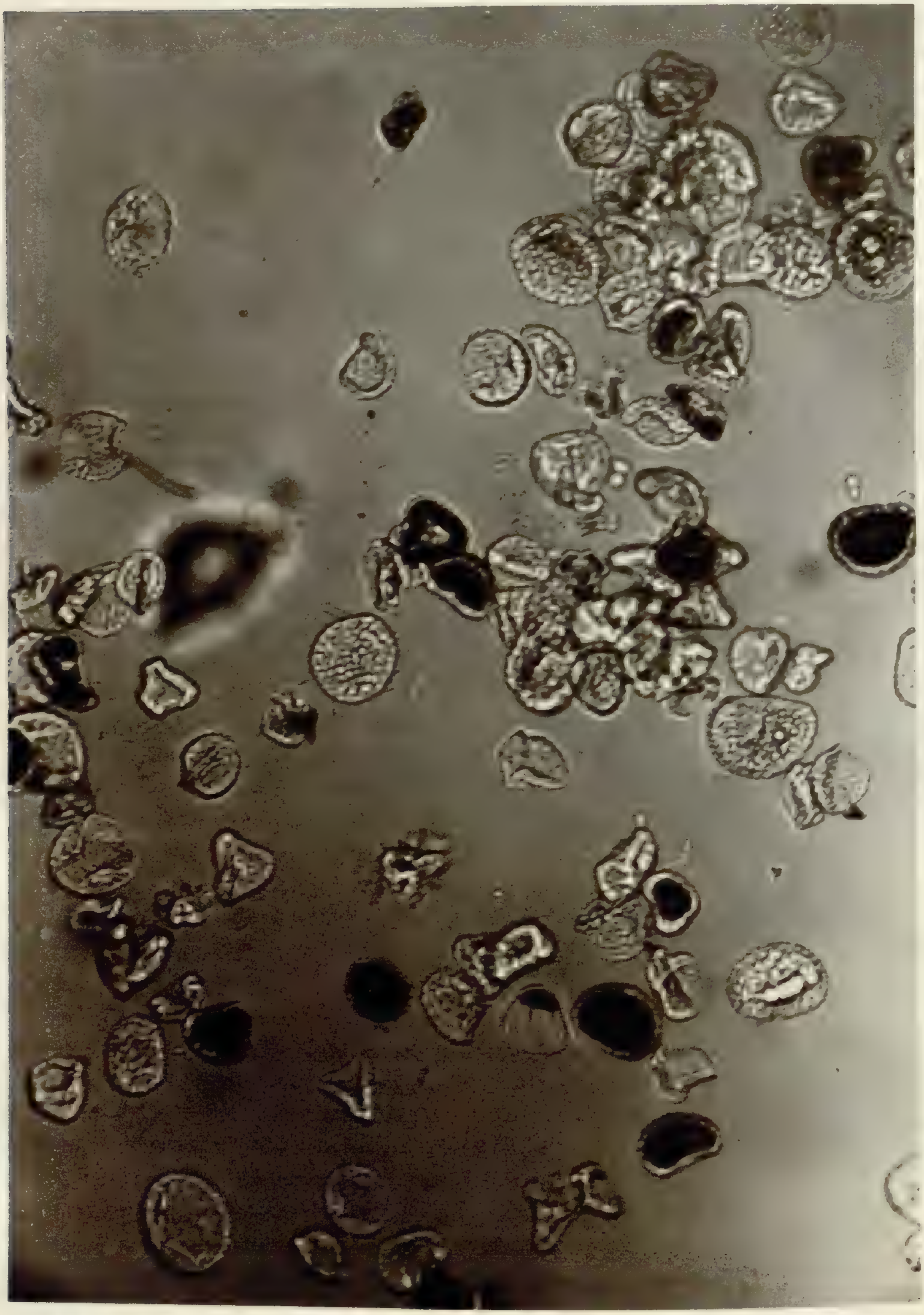


FIG. 1. Light microscope photograph of abortive spores of *Botrychium ascendens* × *crenulatum* Oregon, MICH. Note deviations in spore size, wall collapse, absence of protoplast (evidenced by black appearance). Dwarf and giant spores are especially conspicuous.

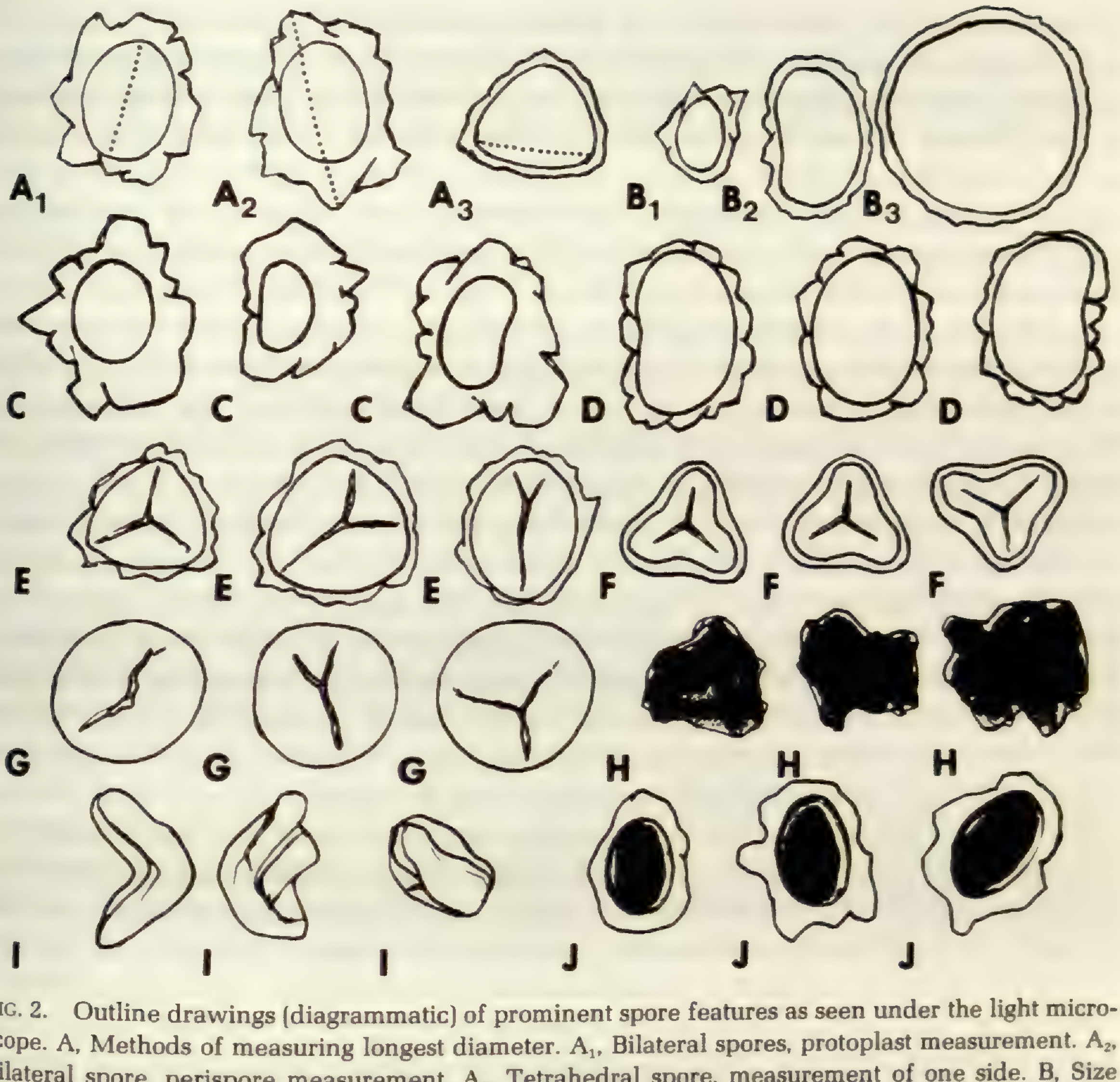


FIG. 2. Outline drawings (diagrammatic) of prominent spore features as seen under the light microscope. A, Methods of measuring longest diameter. A_1, Bilateral spores, protoplast measurement. A_2, Bilateral spore, perispore measurement. A_3, Tetrahedral spore, measurement of one side. B, Size range of single spore sample. B_1, Dwarf. B_2, Normal. B_3, Giant. C, Spores illustrating relationships between development of perispore and protoplast. D, Normal spores to compare with C. E, Apogamous meiospores showing different shapes of laesurae. F, Sexual meiospores showing uniformity of laesurae. G, Diplospores showing round outline and variation of the laesurae (based on *Polypodium dispersum*, Florida). H, Nearly opaque, highly irregular, darkly pigmented abortive spores in which the separate wall layers and protoplast are not visible. I, Collapsed and twisted spores showing concave walls and strongly curved outlines. J, Optical effect of "air pockets" in spores that have lost their protoplasts.

protoplasts as compared to the parents. Major variables that characterize abortion are the following.

Sporangium size.—In some hybrids, the sporangia are smaller than normal, approximately two-thirds or less the size of those of the parents (Wagner & Chen, 1965). Apparently the processes that upset spore development influence the maturation of the whole sporangium. The dwarfed sporangia fail to develop the annulus and stomium completely, and the sporangia do not dehisce. However, mixed with the dwarfed unopened sporangia there may be apparently normal sporangia, even though the spores within are abortive. Thus there is not perfect correlation between abortion of the spores and abortion of the sporangia (De-

Benedictus, pls. 19, 20). Abortion of sporangia seems to be especially frequent in leptosporangiate ferns. One of the most extreme cases of sporangial abortion we have observed occurs in a very rare natural hybrid between *Dryopteris* and *Polystichum* in which the spores are completely deformed. In some years, even at the end of the growing season, no sporangia develop to full size; all are small, closed, and somewhat "crumpled." The capsule walls have not fully differentiated; the annular cells are either entirely unthickened or only sporadically thickened, and their number is considerably reduced from the numbers in annuli normally found in the parents (Wagner & Wagner, unpubl.). Even more severe sporangial abortion was found in *Osmunda claytoniana* × *regalis* (*O.* ×*ruggii*), to the extent that we could not recognize individual spores in the sporangium, the capsule containing coalesced masses of thin-walled cells, and the sporangial walls undifferentiated and fleshy (Wagner et al., 1978).

Deposits on capsule walls.—Sterile hybrids of ferns with typical massive perispores (e.g., *Dryopteris, Polystichum, Asplenium, Blechnum*) often show an irregular development of the perispore in abortive spores, correlated with a deposit of globules of dark material resembling in color the perisporial substance (DeBenedictus, 1969, pl. 22). Although no sporangial abortion per se is detected, this phenomenon usually indicates that something is unusual. It is particularly useful as an indicator in samples of hybrid ferns collected late in the growing season that have discharged all or most of their spores.

Spore size.—Size of spores has two components: dimension of protoplast and dimension of the wall layer. Abortive hybrid spores show much greater variability of size than do normal spores (Figs. 1, 2B). For measuring spores we use a single value, the longest diameter. Tetrahedral spores with very shallow, rudimentary perispore appear triangular under the microscope, the longest dimension being the distance from angle to angle (Fig. $2A_3$). In bilateral spores, the longest dimension is the distance running from end to end as defined by the exospore (Fig. $2A_1$). The latter is complicated, however, by the presence in many bilateral spores of a jagged perispore of considerable thickness. The perispore, however, is usually transparent, so that the measurements can be taken of the outlines of the exospore which is more uniform. The irregular outlines of most perispores lead to an erroneous impression of variability when measured because of the prominent tubercles and ridges (Fig. $2A_2$). In many abortive hybrid spores, unfortunately, it is sometimes difficult to see the exospore (Fig. 2H) and the overall length of the spore must then be used. In this case, comparative studies that include the parents demand that all the spores be measured the same way. Some trilete spores with cristae or equatorial flanges may be difficult to measure and may require measurement of the inner exospore wall. Data from spore sizes may be conveniently plotted as simple frequency histograms (cf. Wagner & Chen, 1965, fig. 4).

Of special interest are the "giant" or "basketball" spores (Figs. 1, $2B_3$) studied extensively by DeBenedictus (1969); these are unreduced spores that have been found to be viable and to produce gametophytes. They are usually circular or elliptical in outline and often lack laesurae. Giant spores are believed to be

unreduced spore mother cells produced by interruptions of meiosis, and often occur only 16 per sporangium in a genus where 64 is the normal number. Perhaps related cytokinetically are the occasional "double" spores (Taylor et al., 1985, pl. 2d) with two units connected by a narrow isthmus. If derived from bilateral spores, the two units, though parallel, are usually attached at one end (De-Benedictus, 1969, pls. 23, 26). If derived from tetrahedral spores, the two units commonly form a centrally constricted structure like a dumbbell or hourglass.

Surface.—The investigation of surface characters received an enormous boost from the advent of the scanning electron microscope (SEM). Unfortunately, in both light microscopic and SEM studies, the observer has to use subjective judgment to pick out and illustrate "representative" or "average" spores. Students are often astonished by the amount of variation that exists in a whole field of spores. For that reason we utilize simple line tracings to illustrate variation in certain spore features (see below). The study of surface patterns in abortive hybrid spores is still in its infancy. Most students of pteridophyte spores have confined themselves to non-abortive spores of normal species. The time is ripe to make detailed SEM studies of hybrid spores to determine the morphogenetic expressions between the parental spore surfaces, i.e., the inheritance pattern of parental spore surfaces in hybrid taxa. For example, what are the spore sculptures of hybrids between species with spinose vs. cristate spores, or verrucate vs. pustulate spores? Examples of hybrid spore surfaces are given by Brooks (1982) and Taylor et al. (1985). The problem with most samples of abortive spores is their extreme variability in all respects, including their outer surfaces. A "typical" or "average" spore surface is thus more difficult to judge than in normal spores. Only by illustrating the range of variations can the spore surfaces of a given intertaxon be completely documented.

Perispore thickness.—SEM does not show the extent of the perispore in comparison with the protoplast, since the latter must be determined by the outline of the exospore which is visible only by light microscopy. Not only do normal species differ in the extent of their respective perisporial thickness and pattern (even between closely related taxa such as *Cystopteris fragilis* and *C. dickieana*), but nothospecies may differ from either of their parents in a transgressive expression of exaggerated perispore material which may or may not be accompanied by a reduction in the size of the protoplast (Fig. 2C vs. 2D). Estimates of the extent of protoplast reduction and perispore exaggeration may be made in SEM only with broken spores. The best way of showing relative perispore/protoplast thickness is by simple line drawings (such as those in W. Wagner, 1966, 1973; Wagner & Boydston, 1958; Fig. 2, A–F, J) which are tracings made with a drawing tube attachment on the compound light microscope. The outer lines of the tracings represent perispores and the inner the approximate borders of the protoplast.

Laesura.—The so-called "scar" of pteridophyte spores takes two different forms: the single straight laesura running along one side of a bilateral spore, producing what is called a monolete spore; and the triradiate laesura of a tetrahedral spore, made up of three rays 120° from each other, producing a trilete spore (Fig. 2F).

There is, in general, close correlation between spore type and the genus of pteridophytes (W. Wagner, 1974). Thus, *Psilotum* has monolete spores and *Botrychium* has trilete spores. In normal species, spores intermediate between these two types are rare (F. Wagner, 1985). In sterile hybrids, as well as apogamous hybrids, however, intermediate laesural forms occur frequently. In Hawaii, the nothospecies *Pteris* ×*hillebrandii,* intermediate between *P. cretica* (apogamous) and *P. irregularis* (sexual) inherits the apogamous characters of *P. cretica*; the hybrid spores, like those of *P. cretica,* are much more diverse in shape than those of the sexual *P. irregularis.* Most interesting are their laesurae: Those of the sexual species are symmetrical and uniform, but those of the apomicts are asymmetrical and variable, running the gamut from trilete to nearly monolete, with the former prevailing (W. Wagner, 1974, fig. 6; this paper, Fig. 2E vs. 2F). Apparent hybridization between *Polypodium atrum* and *P. plumula* has produced the widespread neotropical *P. dispersum.* Possessing an unusual apogamous life cycle, *P. dispersum* is a triploid with 111 unpaired chromosomes but it produces viable spores that are more or less globular with highly variable scar configurations, including monolete and trilete and all forms between (Evans, 1969, fig. 14; this paper, Fig. 2G). Similar spherical and ovoid giant spores in many other hybrids show laesural irregularities similar to those described.

Pigmentation.—Some hybrid abortion is revealed in herbarium specimens simply by scanning the sori with the naked eye. In hybrids they are often blackish or dark brown, in contrast to normal relatives with light brown or tan sori. Further examination under the microscope reveals that the capsules have dark deposits and the spores have exaggerated dark perispores, so strongly pigmented that it is impossible to detect the outlines of the protoplast within (Fig. 2H). Good examples occur in the Appalachian *Asplenium* intertaxa (e.g., Wagner et al., 1973) in which the sori are deep chestnut in color because of the deposits. During the latter half of the growing season, the sori of normal, fertile species often expand to several times their normal width because most of the sporangia are wide open and extend in all directions, and the sori themselves appear to overlap. The sori in most hybrids, in contrast, have so much sporangial abortion that they remain closely contiguous, and this, plus the dark color, distinguishes them from sori of normal species.

Spore collapse.—Collapse of the spore takes the form of major concavities or twisting of the entire exospore, and the whole spore may have a prune-like surface due to depressions (Figs. 1, 2I). Some forms of collapse seem to be associated with absence of the protoplast. Good illustrations of this condition in samples from dried herbarium specimens are seen in the Hawaiian hybrid whiskfern, *Psilotum complanatum* × *nudum* (W. Wagner, 1968, p. 117, fig. 1), and in the Michigan hybrid moonwort, *Botrychium matricariifolium* × *simplex* (W. Wagner, 1980, fig. 3). In the *Psilotum,* spores of the parents are regularly bean-shaped (except for a few abortive ones) and contain protoplasts with storage droplets. The hybrid has many spores with deep concavities, over half of them being empty shells. Spore collapse is probably associated with death of the protoplast because turgor is lost in final development. Drying of the specimen during pressing likely exacerbates the deformity. The technique of staining used

traditionally to test for absence of protoplast in pollen of angiosperms does not work well in most pteridophytes because the spore walls are too thick.

Protoplast.—The protoplast may be influenced in various ways in hybrid sporogenesis. The amount and nature of food storage may be modified, or the entire protoplast, as noted above, may die and be absorbed. In *Lycopodium* sect. *Complanata* [=*Diphasiastrum*], Wilce (1965) discovered that certain interspecific crosses could be recognized by reduced amounts of oil in their protoplasts. Such reduction in oils was correlated with other signs of abortion, e.g., empty or misshapen spores. The massive resistant spore walls had to be broken before she could stain the protoplasts. Protoplast size varies greatly in abortive spores, sometimes reaching notably small dimensions (Fig. $2B_1$). Such minute protoplasts are produced by unequal chromosomal segregation during meiosis, with certain telophase nuclei receiving only a few chromosomes. When abnormal cytokinesis occurs, on the other hand, whole chromosome complements plus the cytoplasm may be doubled, producing giant spores. Some giant spores with huge protoplasts may be more than doubled, perhaps becoming 4*x* or even 8*x*.

Generally, spores lacking a protoplast can be recognized by the absence of any materials inside the exospore; the spore is "blank," as in the *Psilotum* nothospecies discussed above. In herbarium specimens the empty spores are commonly filled with air, producing optically a conspicuous black or dark gray area within the exospore (Figs. 1, 2J). We can say for certain that those spores that lack protoplasts are inviable. We cannot, however, be sure that those spores that have normal-appearing contents are all viable; this can be determined only by experimental germination.

Germinability of spores.—The ultimate test of abortion is viability. Even spores that appear peculiar may germinate if they have not been killed by chemicals or heat for herbarium fumigation (Windham et al., 1986). In some hybrids, the percentage of spores from dried specimens that germinate may be unexpectedly high (DeBenedictus, 1969; Whittier & Wagner, 1971). Some *Dryopteris* hybrids (for example, *D. celsa* × *marginalis* and *D. clintoniana* × *cristata*) have germination of up to one-third of the spores sowed, in spite of great variability in size and other signs of abortion (cf. Whittier & Wagner, 1971, table 1). Among spleenworts such intertaxa as *Asplenium pinnatifidum* × *platyneuron* (*A.* ×*kentuckiense*) and *A. montanum* × *pinnatifidum* (*A.* ×*trudellii*) are notable for their remarkable amounts of germination, probably from giant spores (Wagner & Wagner, unpubl.). DeBenedictus (1969) made a detailed study of a number of "fertile" sterile hybrids, and found that unreduced spores formed gametophytes and that these produced sporophytes apogamously. We believe, therefore, that in spite of their mostly abortive spores, many "sterile" hybrids are capable of forming at least limited populations.

Some Additional Perspectives

Abortive spores of the types described here are best known in homosporous pteridophyte hybrids, especially ferns. But abortion of these or other types also occurs in non-hybrid pteridophytes and in hybrids and non-hybrids of hetero-

sporous pteridophytes and seed plants. Genetic or environmental factors may produce the spore abortion found in some herbarium specimens, the plants involved being normal species and not sterile intertaxa. For example, we are currently studying a wild mutant of *Asplenium platyneuron* with a level of spore abortion that exceeds that of the normal form that grows with it. The mutant also has a tendency for 32-spored sporangia (Wagner & Wagner, unpubl.). Spore abortion also within species is seen in the occasional triploids in which the process of meiosis is confused by the presence of three rather than two chromosome sets. However, Haufler et al. (1985) determined that in triploid *Cystopteris protrusa* numerous normal-appearing spores occur, in addition to spores that are deformed, making a triploid diagnosis difficult. Hybrids between diploid and tetraploid varieties of the same species produce abortive spores, e.g., triploid *Asplenium trichomanes* in the eastern United States and Canada. The use of normal vs. abortive spores and small vs. large spores in herbarium material was nicely illustrated by Moran (1982), who was able to describe the geographical distributions of the 2*x*, 3*x*, and 4*x* forms of *A. trichomanes*, the first with small spores, the second with abortive spores, and the third with large spores.

Another non-hybrid source of abortion may be cold or drought shocks during spore development. Wilce (1965) stated that "In *Lycopodium alpinum, L. sitchense*, and *L. complanatum*, the relatively high rate of abortion is perhaps accounted for by the northern distribution of these species, for abortive spores and pollen are not uncommon in other plants of high latitudes." She also suggested that some spore abortion in what appear to be normal species may be due to backcrossing that produces plants so closely resembling one of the parents that they cannot be distinguished. In a monograph of the genus *Pyrrosia*, Hovenkamp (1986) reported collections with abortive spores in nearly three dozen taxa, all of which also have collections with normal spores.

That unusual patterns of abortion occur in hybrids of certain heterosporous pteridophyte groups is nicely illustrated by the hybrids of the quillwort genus *Isoëtes*. Extensive recent studies by one of us (Taylor) indicate that hybridization is much more common than previously realized. The hybrids are recognized by their production of flattened and polymorphic megaspores of which less than 1% germinate in culture. Hybrid *Isoëtes* show two types of megaspore abortion. In diploid hybrids, megaspores are seen to be relatively uniform in size and shape but they appear small and flattened (especially on their proximal sides). In hybrids of higher ploidy, spore abortion is characterized by the production of polymorphic megaspores. While there continues to be production of numerous flattened megaspores like those found in the diploids, there are also many larger, irregularly shaped megaspores. Some of these larger megaspores are hemispherical, others are found to be fusiform, and a few are even dumbbell-shaped when two spores are linked by an isthmus. Occasionally, an entire tetrad will be found that is interconnected by isthmuses. Further, the triradiate ridges that typically mark the proximal side of the megaspore are usually irregularly formed. In addition to variation in spore shape and triradiate ridge form, megaspore dimensions may range from less than 100 μm to over 1000 μm within a single sporangium.

Although some of the larger quillwort megaspores open and exude a pad of gametophyte tissue, the few spores that germinate to produce gametes with normal-looking archegonia are not the abnormally large "basketball" spores described for homosporous pteridophytes but globose, average-sized megaspores which occur in very low numbers. It appears that the globose, more normal-looking megaspores possess an ornamentation pattern intermediate between putative parents. Microspore abortion is also evident in hybrid taxa. Abortive microspores are irregular in shape and most appear to be devoid of cytoplasm.

We should mention here the situation so common in flowering plants, in which the pollen grains may be quite uniform in size and shape but lack protoplasts. Thus it is possible to take masses of pollen grains, stain them, and determine which grains have and which have no protoplasts. Those that do not take stain lack protoplasts. Seed plant hybrids tend to have high percentages of empty pollen grains, and normal species have low percentages.

We believe that one of the primary functions of surveys of herbarium collections of pteridophytes for spore abortion is to form testable hypotheses regarding hybridity. The coincidence of interspecific intermediacy together with abortive spores is a sure sign of hybridity at least in homosporous pteridophytes; this combination of circumstances has occurred so commonly in biosystematic research over the past thirty years that likelihood of any other explanation is exceedingly small. However, abortion may sometimes be due to other factors than hybridity, as pointed out here. We hope that in the future more will be learned about the comparative aspects of abortion. For example, are the patterns of abortion different in different intertaxa? Is hybrid abortion different from intraspecific? The fact that we find such strong differences between groups such as homosporous and heterosporous pteridophytes and between non-seed plants and seed plants suggests that there are important differences in sporogenesis that characterize these groups. We can learn about these questions only by acquiring more and better data than we have now.

Literature Cited

Anderson, L. W. 1954. Hoyer's solution as rapid permanent mounting medium for bryophytes. Bryologist 57:242–244.

DeBenedictus, V. M. M. 1969. Apomixis in ferns with special reference to sterile hybrids. Ph.D. Dissertation, University of Michigan.

Brooks, R. E. 1982. A new Woodsia hybrid from Kansas. Amer. Fern J. 72:79–84.

Evans, A. M. 1969. Interspecific relationships in the *Polypodium pectinatum-plumula* complex. Ann. Missouri Bot. Gard. 55:193–283.

Haufler, C. H., M. C. Windham, D. M. Britton, and S. J. Robinson. 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. Canad. J. Bot. 63:1855–1863.

Hauke, R. L. 1963. A taxonomic monograph of the genus *Equisetum* subgenus *Hippochaete*. Beih. Nova Hedwigia 8:1–123, tables 1–12, graph 1–3, pl. 1–22.

Hovenkamp, P. 1986. *A monograph of the genus* Pyrrosia. Brill/Leiden Univ. Press.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: University Press.

Moran, R. C. 1982. The *Asplenium trichomanes* complex in the United States and adjacent Canada. Amer. Fern J. 72:5–11.

TAYLOR, W. C., N. T. LUEBKE, and M. B. SMITH. 1985. Speciation and hybridization in North American Quillworts. Proc. Royal Soc. Edinburgh 86B:259–263.

WAGNER, F. S. 1985. Bilateral spores in New World grammitid ferns. Amer. Fern J. 75:6–11.

WAGNER, W. H. Jr. 1966. New data on North American oak ferns, *Gymnocarpium*. Rhodora 68: 121–134.

———. 1968. Hybridization, taxonomy, and evolution. Chapt. 9 in *Modern methods in plant taxonomy*, ed. V. H. Heywood. London: Academic Press.

———. 1973. Reticulation of holly ferns (*Polystichum*) in the western United States and adjacent Canada. Amer. Fern J. 63:99–115.

———. 1974. Structure of spores in relation to fern phylogeny. Ann. Missouri Bot. Gard. 61:332–353.

———. 1980. A probable new hybrid grapefern, *Botrychium matricariifolium* × *simplex*, from central Michigan. Michigan Bot. 19:31–36.

———. 1983. Reticulistics: The recognition of hybrids and their role in cladistics and classification. Chapt. 8 in *Advances in cladistics*. Vol. 2, eds. N. I. Platnick and V. A. Funk. New York: Columbia Univ. Press.

——— and K. E. BOYDSTON. 1958. A new hybrid spleenwort from artificial cultures at Fernwood and its relationship to a peculiar plant from West Virginia. Amer. Fern J. 48:146–159.

——— and K. L. CHEN. 1965. Abortion of spores and sporangia as a tool in the detection of *Dryopteris* hybrids. Amer. Fern J. 55:9–29.

———, F. S. WAGNER, J. A. LANKALIS, and J. F. MATTHEWS. 1973. *Asplenium montanum* × *platyneuron*. A new primary member of the Appalachian spleenwort complex. J. Elisha Mitchell Sci. Soc. 89:218–223.

———, ———, C. N. MILLER, JR., and D. H. WAGNER. 1978. New observations on the royal fern hybrid, *Osmunda* × *ruggii*. Rhodora 80:92–106.

WHITTIER, D. P. and W. H. WAGNER, Jr. 1971. The variation in spore size and germination in *Dryopteris* taxa. Amer. Fern J. 61:123–127.

WILCE, J. H. 1965. Section *Complanata* of the genus *Lycopodium*. Beih. Nova Hedwigia 19:i–ix, 1–233, pl. 1–40.

WINDHAM, M., P. G. WOLF, and T. A. RANKER. 1986. Factors affecting prolonged spore viability in herbarium collections of three species of *Pellaea*. Amer. Fern J. 76:141–148.

American Fern Journal 76(3):141–148 (1986)

Factors Affecting Prolonged Spore Viability in Herbarium Collections of Three Species of *Pellaea*

MICHAEL D. WINDHAM, PAUL G. WOLF, and THOMAS A. RANKER
Department of Botany, University of Kansas, Lawrence, Kansas 66045

Among lower vascular plants, the establishment of new individuals and populations is largely dependent on the transport of spores by prevailing winds (Tryon, 1970; Wagner, 1972). Since long distance dispersal may require prolonged exposure of spores to unfavorable conditions, spore longevity has a major impact on species migration and distribution. The degree to which spores remain viable for long periods also determines the applicability of many biosystematic techniques to herbarium specimens (Windham & Haufler, 1986). Although an understanding of spore longevity is thus critical to investigations of several biological phenomena, little is known about the factors affecting prolonged spore viability in ferns.

Previous studies have shown that the spores of various pteridophyte species differ greatly in their ability to germinate after storage at ambient temperatures (Dyer, 1979). Two major groups of ferns have been identified on the basis of spore longevity (Lloyd & Klekowski, 1970): those with green spores containing active chlorophyll (mean viability of 48 days), and those with non-chlorophyllous spores (mean viability of 2.8 years). Most studies of the factors affecting spore longevity have compared these two groups. For example, Lloyd & Klekowski (1970) suggested that high water content, elevated respiratory rates, and the absence of a desiccation-resistant spore wall might account for the short viability of chlorophyllous spores. Since viability declines rapidly in *Equisetum* spores even when they are protected from desiccation (Hauke, 1963), the intensity of respiratory activity seems to be the limiting factor in this case. Although these studies may explain the lack of long-term viability in green spores, they shed little light on factors controlling longevity in the majority of fern taxa with non-green spores.

Depending on the species, non-chlorophyllous fern spores remain viable for periods ranging from several months (Lloyd & Klekowski, 1970) to nearly 100 years (Johnson, 1985). Both temperature and humidity are known to influence longevity (Okada, 1929), but a variety of other factors may also be involved. Although differences in respiratory rate seem relatively minor in this group (Okada, 1929), they may prove significant when calculated in terms of years or decades. Thus, ploidy level could be an important factor because polyploids tend to show lower respiratory rates (Levin, 1983). In addition, the lower surface/volume ratios of polyploid spores would reduce the relative exposure of cytoplasm to the unfavorable environment surrounding the spore. For herbarium specimens traditionally used in spore longevity studies, this unfavorable environment may include a variety of physical and chemical treatments used for preservation and insect control. All of these factors have the potential to affect

spore longevity, and their relative importance should be assessed through comparative studies of germination in a variety of non-chlorophyllous fern spores.

The fern genus *Pellaea* provides a good starting point for such investigations. Many members of this genus are xerophytic, and Pray (1968) indicated that the spores of most specimens remain capable of germination 10–15 years after pressing. In addition, Mickel (1962, pers. comm.) reported that a spore collection of *Pellaea atropurpurea* (L.) Link yielded viable gametophytes after 34 years, one of the oldest germination records among homosporous ferns. Several taxa (and ploidy levels) are common in the United States, where they have been heavily collected through the years and are well-represented in both large and small herbaria. The present study examines the germination ability of spores derived from herbarium specimens of three closely related species: *P. truncata* Goodd., *P. wrightiana* Hook., and *P. ternifolia* (Cav.) Link. Germination patterns are then compared with data on ploidy level, specimen age, and herbarium treatment to elucidate some of the factors affecting spore longevity in *Pellaea*.

Materials and Methods

Collections from the following ten herbaria were included in the study after obtaining explicit permission from the respective curators (abbreviations are those of Holmgren et al., 1981): ARIZ, ASU, COLO, MICH, MO, MSC, NY, UC, UNM, and US. For each herbarium, one sporulating specimen of *Pellaea truncata* (diploid) and *P. wrightiana* (tetraploid) was chosen to represent each decade from the 1930s to the 1980s. When collections of these species were unavailable, the appropriate cytotypes of *P. ternifolia* were substituted. Spores from all samples were measured at 400× using an ocular micrometer mounted on a Leitz compound microscope, and ploidy levels were then assigned on the basis of established correlations between chromosome number and spore length (Tryon, 1968; Windham, unpubl.). The effect of ploidy level on prolonged spore viability was tested using the paired collections of haploid and diploid spores from each herbarium. Although it would have been desirable to base this comparison solely on the cytotypes of a single autopolyploid species (such as *P. ternifolia*), this approach was rendered impossible by the small number of specimens available. The allotetraploid *P. wrightiana* was derived through hybridization between *P. truncata* and *P. ternifolia* (Wagner, 1965), and it was hoped that genetic similarities among these taxa would minimize the effect of interspecific differences on the paired ploidy level comparisons.

Spores were cultured on an inorganic medium enriched with Parker's macronutrients and Thompson's micronutrients and solidified with 1% agar (Klekowski, 1969). The medium was autoclaved and poured into sterilized plastic petri dishes (35 mm diameter) which were kept inside clear plastic boxes throughout the experiment to prevent excessive water loss. Plants chosen as spore sources were lightly dusted with compressed air prior to sowing to reduce the possibility of contamination by stray spores. Spores were sown by carefully inverting a sporulating section of an herbarium specimen directly over a petri dish and lightly tapping the sheet to deliver the desired quantity of spores (ca.

1000 per dish where available). The cultures were maintained under Sylvania Gro-Lux bulbs on a 12 hour photoperiod at an irradiance of 73 microeinsteins/m^2/sec. (PAR) and a temperature of 22 (±2)°C.

Germination was defined as the first emergence of the gametophyte from the ruptured spore wall since this is the most reliable measure of physiological activity in the absence of ultrastructural or biochemical data (Miller, 1968). The condition of the spores was checked every three days for most collections. Preliminary data gathered at three-day intervals suggested that specimen age might be positively correlated with the amount of time required to germinate. Since COLO and UNM provided the most complete temporal series of germinating spores, collections from these herbaria were resown and observed daily to determine the strength of the correlation. Sample size was increased in this analysis by incorporating several specimens that were not part of the original study. Spearman's rank correlation coefficients (r_s; Spearman, 1904; Zar, 1972) were calculated to test for significant associations between age of specimen and number of days from sowing to germination. A spore collection was considered viable when more than 1% of the spores in a petri dish showed signs of gametophytic emergence. Collections showing less than 1% germination were considered dead since the possibility of contamination by extraneous spores could not be ruled out. All germination ceased during the fifth week after sowing, and observations were discontinued after seven weeks. Percent germination was estimated at the end of the experiment by determining whether viable gametophytes outnumbered ungerminated spores. A complete list of the specimens used in this study is available from the senior author upon request.

Results

Several patterns emerge from the spore germination data (Table 1), the most obvious of which involve differences in viability between herbaria. All specimens from ASU and COLO, and all but one from UNM, produced spores capable of germination. All collections from MO, UC, and US failed to germinate, and germination was observed in only a single specimen from MICH. At the remaining three herbaria (ARIZ, MSC, and NY), only relatively recent collections (post 1940s) germinated. Low germination percentages in five of the six oldest collections provided additional evidence of the predicted negative correlation between spore viability and specimen age. However, the rate of decline in germination ability was much lower than expected. Eleven of the 46 specimens (24%) collected prior to 1960 showed spore germination, and one plant of *Pellaea truncata* collected in 1936 (*Craig* s.n., UNM) still had viable spores.

Although spores from both COLO and UNM remained viable for nearly 50 years, the two herbaria showed clear differences in germination rate. All spores from COLO germinated in six to ten days, whereas samples from UNM required five to 25 days to germinate. In the sample from COLO, the number of days to germinate was not associated with the age of the specimen ($r_s = 0.470$, $0.10 < P < 0.15$). In contrast, there was a highly significant positive association between time to germination and age of specimen in cultures from UNM ($r_s = 0.969$, $P <$

TABLE 1. Variability in Spore Germination Among Herbarium Specimens of *Pellaea*.

Herbarium	1980s		1970s		1960s		1950s		1940s		1930s	
	2×	4×	2×	4×	2×	4×	2×	4×	2×	4×	2×	4×
ARIZ	++	+	++	++		−[1]	+	−	−	−	−	−
ASU	++	++	++	++	++	++			+			
COLO	+[1]		++	++	++[1]	++	++		++		+	
MICH			−	++[1]	−	−	−	−[1]	−	−	−[1]	−
MO	−[1]	−[1]		−[1]		−[1]	−	−	−		−	−
MSC	++		−	++	++	++	−[1]	++			−[1]	
NY	++	++	+	+	−		−[1]		−	−	−	−
UC			−		−	−	−	−	−	−	−	
UNM			+	++	+	++	+	++	+	+	+	−
US			−		−		−	−	−	−	−	−

[1] The appropriate cytotype of *Pellaea ternifolia* was substituted since collections of *P. truncata* or *P. wrightiana* were not available.

++ 51–100% germination; + 1–50% germination; − no germination.

0.001). Although the data are incomplete and less precise, it appears that all other herbaria with viable spores exhibit patterns of association similar to that recorded for UNM. Thus, the lack of correlation between germination rate and specimen age at COLO may be considered exceptional.

In order to test the association between ploidy level and spore longevity, 36 paired spore collections were examined. Each pair consisted of haploid and diploid samples collected within ten years of one another and housed in the same herbarium. Five (14%) of these showed germination of only one member of the pair (Table 1). Failure to germinate appeared to be a random phenomenon with respect to ploidy level since the diploid showed greater longevity in two cases and the haploid in three. A chi-square test of the entire data matrix revealed that haploid and diploid spores did not differ in their ability to germinate through time ($\chi^2 = 0.002$, $P > 0.9$, for a 2×2 contingency table of ploidy [haploid or diploid] vs. germination [yes or no]).

DISCUSSION

Ploidy level.—At the outset of this study, it was predicted that spore longevity should be more prevalent at higher ploidy levels since polyploids tend to show lower respiratory rates and have larger spores with lower surface/volume ratios. Of the seven fern taxa previously reported to germinate after 25 years (Allsopp, 1952; Fischer, 1911; Johnson, 1985; J. Mickel, 1962, pers. comm.), two (*Asplenium serra* Langsd. & Fisch. and *Pellaea atropurpurea* (L.) Link) are definitely polyploid (Löve et al., 1977). The remaining five species belong to the genus *Marsilea*, for which few chromosome counts are available. Even if all of the *Marsilea* species were diploid, they would not be comparable to diploid homosporous ferns due to the large size of the megaspores and the presence of a protective sporocarp. Thus, on the basis of these limited data, it might be argued that the

spores of polyploids do show greater longevity. However, analyses of the 90 specimens included in the present study do not support the predicted relationship between ploidy level and spore longevity. Although diploid plants from UNM tend to show lower germination percentages than tetraploids (Table 1), this pattern was not observed in other herbarium collections and long-term viability was apparently unaffected. Overall, the oldest germinating spores were derived from the diploid species *Pellaea truncata* (Table 1), but the chi-square test verified that germination ability was independent of ploidy level.

Specimen age.—In herbaria showing some germination activity, percent germination tended to decline through time, eventually culminating in a complete loss of viability (Table 1). Occasional deviations from this trend probably can be explained in light of the unique history of each specimen. For example, the tetraploid 1950s collection from MSC showed greater than 50% germination whereas the diploid sample from that decade failed to germinate entirely. It therefore seems likely that one of these plants did not follow the normal sequence of events involved in curation at MSC. Further investigation has shown that the tetraploid specimen (*Cobean s.n.*) was derived from a plant in the University of California Botanical Garden which was still alive and providing chromosome counts in the mid-1960s (Lloyd, 1966). Thus, although this plant was collected in 1956, it probably wasn't pressed and incorporated into the herbarium until the 1960s, and the germination response reflects this fact. If the history of each specimen included in this study were known, it is quite possible that most of the anomalies in the germination records would disappear. Regardless of such individual idiosyncrasies, the overall pattern of germination remains relatively clear and interpretable.

The prolonged retention of spore viability in these *Pellaea* collections was somewhat unexpected. Twenty-four percent of specimens older than 25 years remained capable of germination, and the spores of one plant were viable after 50 years. The 1930s were arbitrarily chosen as the early limit of this investigation based upon a small pilot study which yielded no germination prior to 1936. However, most of the pre-1930 specimens included in that study were derived from MO and US, which showed no evidence of germination even in recent collections. Therefore, it is quite possible that further research using older specimens from ASU, COLO, or UNM will produce new records for spore longevity in *Pellaea*.

Herbarium treatment.—The number of days from sowing to germination increased as the age of the specimen increased in most herbarium collections, and UNM provides a clear example of this trend. The only clear exception is COLO, which shows no association between time to germination and date of collection. This may be explained by the fact that the University of Colorado herbarium is situated in a cool, dry environment and has never been forced to use poisons or insect repellents (W. Weber, in litt.). Thus, instead of being a natural consequence of aging, slower germination may be caused (or at least accentuated) by treatments designed to keep the herbarium free of destructive insects. In fact, it appears that most of the differences in germination among herbaria may be a result of different curatorial techniques.

All collections from MO and US were subject to repeated or continuous exposure to methyl bromide or Dowfume, both of which are listed by the Environmental Protection Agency as highly toxic (Category I) poisons (Schofield & Crisafulli, 1980). Judging from the fact that specimens from MO and US showed no germination, it is likely that these two insecticides are just as deadly to fern spores as they are to insects and humans. In the late 1970s, NY discontinued the use of Dowfume and substituted pyrethrum, which is effective against insects but harmless to mammals (Schofield & Crisafulli, 1980). The pronounced increase in spore viability at NY during this period may be a consequence of this change in curatorial practices. Chemical treatments do not represent the only threat to spore longevity in herbarium collections. All specimens at UC were heat-treated at least once at 60°C for 24 hours, apparently resulting in a complete loss of viability. MICH and ARIZ have used a combination of insecticides through the years, including heat, *para*-dichlorobenzene (PDB), and (at the latter herbarium) carbon bisulfide or carbon tetrachloride. This may account for the sporadic germination patterns observed in collections from these herbaria. Until 1980, the sole insecticide used at ASU was PDB, after which time all incoming and outgoing specimens were frozen for about four days at −20°C. These techniques appear to have little or no effect on spore viability since all collections from this herbarium germinated. UNM and MSC used identical treatments (PDB only), but the latter herbarium showed reduced spore longevity. Higher humidity at Michigan State University could be responsible for this loss (see Dyer, 1979), and it may be significant that spore viability increased at MSC in the 1960s when the herbarium was first air-conditioned. Percentage and rate of germination were highest at COLO, where prevailing environmental conditions make insecticides unnecessary.

Botanists engaged in biosystematic studies of ferns have traditionally underestimated the research value of herbarium specimens. Now that this potential is being realized, investigators should do everything possible to insure that herbarium resources are not depleted or misused. Since viable spores are especially useful in biosystematic research (Windham & Haufler, 1986), efforts should be made to identify and implement curatorial techniques that maximize spore longevity in herbarium collections. Obviously, most herbaria cannot follow the example of COLO, or they soon would be overrun with insects. However, freezing of specimens has proven effective in insect control, and exposure to temperatures of −20°C seems to have no ill effects on spore viability. Although long-term impacts cannot be assessed at this time, it appears that the insecticide pyrethrum (in the form of Dione powder) may have little impact on viability. It is clearly preferable to the use of Dowfume, methyl bromide, and heat treatments, which quickly result in loss of viability and should be discontinued if at all possible. None of the herbaria included in this study use microwave radiation, but this should also be added to the list of treatments that are likely to reduce spore longevity. Although effective against insects, microwave exposure disrupts the internal cell structure of pressed plants (Bacci et al., 1985) and has been shown to reduce seed viability in herbarium specimens of Malvaceae (Hill, 1983).

Aside from encouraging curators to experiment with less destructive means of

insect control, individual botanists can increase the future research value of their own collections by depositing duplicates in herbaria where such treatments are already in use. A comprehensive list of treatments used by each herbarium could be included in future editions of *Index Herbariorum* and would be useful to collectors and biosystematists alike. For their part, researchers intending to use herbarium specimens for biosystematic studies should fully describe their techniques to the curator, obtain permission in advance, and carefully follow all curatorial instructions. Through close cooperation among collectors, herbarium personnel, and biosystematists, the research value of herbarium collections can be greatly enhanced.

We thank the following curators for granting permission to use their specimens in the germination study and for providing information concerning curatorial techniques: Dr. John Beaman (MSC), Dr. David Lellinger (US), Dr. William Martin (UNM), Dr. Charles Mason (ARIZ), Dr. John Mickel (NY), Dr. Nancy Morin (MO), Dr. Donald Pinkava (ASU), Dr. Alan Smith (UC), Dr. Warren H. Wagner, Jr. (MICH), and Dr. William Weber (COLO). We also thank Dr. Christopher Haufler for reading the original manuscript and providing helpful comments and criticisms.

Literature Cited

Allsopp, A. 1952. Longevity of *Marsilea* sporocarps. Nature 169:79–80.

Bacci, M., A. Checcucci, G. Checcucci, and M. Palandri. 1985. Microwave drying of herbarium specimens. Taxon 34:649–653.

Dyer, A. 1979. The culture of gametophytes for experimental investigation. Pp. 253–305 in *The experimental biology of ferns*, ed. A. Dyer. London: Academic Press.

Fischer, H. 1911. Licht- und Dunkelkeimung bei Farnsporen. Beih. Bot. Centralbl. 27:60.

Hauke, R. 1963. A taxonomic monograph of the genus *Equisetum*, subgenus *Hippochaete*. Nova Hedwigia 8:1–123.

Hill, S. 1983. Microwave and the herbarium specimen: potential dangers. Taxon 32:614–615.

Holmgren, P., W. Keuken, and E. Schofield. 1981. Index herbariorum. Part I. 7th ed. Regnum Veg. 106:1–452.

Johnson, D. 1985. New records for longevity of *Marsilea* sporocarps. Amer. Fern J. 75:30–31.

Klekowski, E. 1969. Reproductive biology of the Pteridophyta III. A study of the Blechnaceae. J. Linn. Soc., Bot. 62:361–377.

Levin, D. 1983. Polyploidy and novelty in flowering plants. Amer. Naturalist 122:1–25.

Lloyd, R. 1966. Pteridaceae. In IOPB chromosome number reports VIII, ed. A. Löve. Taxon 15: 282–283.

——— and E. Klekowski. 1970. Spore germination and viability in Pteridophyta: Evolutionary significance of chlorophyllous spores. Biotropica 2:129–137.

Löve, A., D. Löve, and R. Pichi Sermolli. 1977. *Cytotaxonomical atlas of the Pteridophyta*. Vaduz: Cramer.

Mickel, J. 1962. A monographic study of the fern genus *Anemia*, subgenus *Coptophyllum*. Iowa State Coll. J. Sci. 36:349–482.

Miller, J. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–440.

Okada, Y. 1929. Notes on the germination of the spores of some pteridophytes with special regard to their viability. Sci. Rep. Tôhoku Imp. Univ., Ser. 4, Biol. 4:127–182.

Pray, T. 1968. The gametophytes of *Pellaea* section *Pellaea*: Dark-stiped series. Phytomorphology 18:113–142.

Schofield, E. and S. Crisafulli. 1980. A safer insecticide for herbarium use. Brittonia 32:58–62.

SPEARMAN, C. 1904. The proof and measurement of association between two things. Amer. J. Psychology 15:72–101.

TRYON, A. 1968. Comparisons of sexual and apogamous races in the fern genus *Pellaea*. Rhodora 70:1–24.

TRYON, R. 1970. Development and evolution of fern floras of oceanic islands. Biotropica 2:76–84.

WAGNER, W. Jr. 1965. *Pellaea wrightiana* in North Carolina and the question of its origin. J. Elisha Mitchell Sci. Soc. 81:95–103.

———. 1972. Disjunctions in homosporous vascular plants. Ann. Missouri Bot. Gard. 59:203–217.

WINDHAM, M. and C. HAUFLER. 1986. Biosystematic uses of fern gametophytes derived from herbarium specimens. Amer. Fern J. 76:114–128.

ZAR, J. 1972. Significance testing of the Spearman rank correlation coefficient. J. Amer. Statist. Assoc. 67:578–580.

American Fern Journal 76(3):149–159 (1986)

Systematic Inferences from Spore and Stomate Size in the Ferns

DAVID S. BARRINGTON and CATHY A. PARIS
Botany Department, University of Vermont, Burlington, Vermont 05405-0086
THOMAS A. RANKER
Botany Department, University of Kansas, Lawrence, Kansas 66045

Size of equivalent cells has traditionally been assumed to be constant within species and variable between species. Systematists have commonly measured cells with constant form, such as spores and stomates, as a means of distinguishing species and hybrids in polyploid complexes, since the best known factor determining cell size is ploidy level (Stebbins, 1950). Preliminary hypotheses about polyploid evolution can be generated directly from herbarium specimens based on spore and stomate measurement data.

The first evidence of a relation between cell size and polyploidy in the ferns was Lawton's 1932 demonstration that prothallial cells, lower epidermal cells, and stomates in induced apogamous races of *Dryopteris marginalis* and *Woodwardia virginica* vary directly with ploidy level. Butters and Tryon's 1948 work on *Woodsia* ×*abbeae* included epidermal cell and annulus cell measurements that corroborated data from spore germination experiments to document an instance of somatic autopolyploidization.

More recently a considerable body of evidence has been assembled supporting the contention that spore size is related to ploidy level in the ferns. Manton (1950) noted that Vancouver Island *Cystopteris fragilis* ($n = 84$) has smaller spores than Swiss *Cystopteris alpina* ($n = 126$). Hagenah (1961) postulated a polyploid series in species of *Cystopteris* based on spore-size measurements. In a monograph of *Cystopteris*, Blasdell (1963) concluded that it was possible to infer ploidy level from spore size in that genus. Blasdell assigned ploidy levels to cytologically unknown components of polyploid complexes based only on spore size (see Lovis, 1977 for a critique). Wagner (1966) demonstrated that there were two varieties of *Gymnocarpium dryopteris*: a larger-spored typical variety, which is tetraploid, and a smaller-spored variety *disjunctum*, which is diploid. Schneller (1974) recently provided an analysis of ploidy level and spore size in the *Dryopteris filix-mas* group of Europe. He concluded that ploidy and spore size are positively correlated for diploid to pentaploid cytotypes in a closely knit phylogenetic group.

In an exhaustive analysis of spore features of northeastern North American *Isoetes*, Kott and Britton (1983) provided new insight into spore-size variability. Whereas megaspore variation within sporangia of a single plant, between sporangia of a single plant, and between plants of a population was found to be more or less equivalent and negligible, variation between populations of a species was demonstrated to be twice that between plants of a population. Microspores were found to be slightly less variable. Kott and Britton (1983) suggested that

spore size can be used to characterize species so long as a sample of at least 20 spores be measured from single plants of a representative set of populations. They demonstrated that, between species, both microspore and megaspore sizes varied directly with ploidy level.

Some spore-measurement analyses have yielded confusing results. Brown (1964) found that spores of the tetraploid *Woodsia oregana* var. *cathcartiana* were the same size or smaller than those of var. *oregana*, which is diploid. His results, however, are apparently a consequence of having mixed diploid and tetraploid cytotypes of variety *oregana* (see Windham & Haufler, 1986). More intriguing are the apparently inconsistent results for *Dryopteris*. Manton (1950) found it odd that spores of diploid *Dryopteris abbreviata* and tetraploid *D. filix-mas* were almost identical in size. Britton (1968) concluded from an analysis of eastern North American *Dryopteris* species that spore size and ploidy level are not closely correlated in the genus, based on documentation of relatively large spore sizes of the diploid species *D. fragrans* and *D. dilatata* (=*D. expansa*). He found that mean spore size of *D. dilatata* (=*D. expansa*) was indistinguishable from that of the tetraploids *D. campyloptera* and *D. spinulosa*. Wagner (1971) provided spore-length data for an expanded set of *Dryopteris* species in his survey of Appalachian wood ferns. His data corroborate Britton's observation that there is no simple relationship between spore length and ploidy level in *Dryopteris*.

The observations on *Dryopteris* by Manton, Britton, and Wagner suggest that sizes of diploid spores within a genus can be quite different. This variation in spore size among diploid species depends on at least three factors. 1) Size varies with adaptation for dispersal. Small size increases the likelihood of dispersal outside the region of the parent, but spore size increases on islands so that propagules can remain on the island rather than disperse into terrain that cannot host gametophyte development and function (Carlquist, 1966). 2) Spore size is also thought to increase for nutritional reasons (as in the evolution of megaspores). 3) Cox and Hickey (1984) provided evidence that environmental parameters strongly affect spore size in *Isoetes storkii* of Costa Rica. Their investigation of three populations whose habitats differed in mean temperature, mean daily solar radiation, and altitude demonstrated that plants in colder, shadier, higher habitats had smaller spores. This paper suggests that analyses of cell size variation among species should, when possible, include a representative sample of populations from a set of characteristic habitats for each species. Hence, spore size is determined not only by ploidy level but by the reproductive biology and environmental regime of a taxon.

Stomate size (usually as length of the guard cells) has also been correlated with ploidy level. Wagner (1954) noted that tetraploid members of the Appalachian *Asplenium* complex had larger stomates than the diploids. Lovis and Reichstein (1968) provided data on stomate length for two sterile diploid hybrids and each of their derived fertile tetraploids. In each case the tetraploid stomates are longer than those of the corresponding diploid, and there is little overlap of diploid and tetraploid stomate samples. Schneller (1974) reported stomate lengths for members of the *D. filix-mas* complex in Europe. He demonstrated a correlation between ploidy level and stomate size for diploid to pentaploid cytological

races. Stomate measurements are particularly useful in assessing sterile hybrids, since irregular spores preclude spore comparisons.

Counterintuitive results of stomate-size analysis have also been reported. Barrington (1986) reported stomate lengths of diploid *Polystichum acrostichoides*, tetraploid *P. braunii*, and their hybrid, which is known as both triploid and tetraploid. The order of these taxa based on stomate length (smallest to largest) was: tetraploid progenitor, triploid hybrid, diploid progenitor, tetraploid hybrid. Barrington suggested that *P. braunii* was derived from diploid Asian species with much smaller stomates than those of *P. acrostichoides*, but could not explain the anomalous guard-cell sizes of the hybrids. We provide an analysis of the anomalous stomate sizes in this hybrid below.

Experimental error in measurement has been a problem in the work with spore and stomate size. Factors affecting the measurement of cells include: 1) calibration of microscope; 2) characteristics of mounting medium; 3) definition of cell boundary; and 4) choice of cells for measurement. Lovis (1964) encountered a 30% discrepancy between his data sets and those of Meyer in spore-size measurements of the European *Asplenium trichomanes* complex. Difference in mounting medium accounted for 6% of the difference: Lovis found that his spores, in gum chloral, yielded measurements 6% higher than Meyer's spores of the same taxon, which were measured dry. Lovis also determined that Meyer's measuring all spores versus his measuring only those whose long axis was perpendicular to the line of sight accounted for a further 1.5–3.5% discrepancy. Nevertheless, he was unable to account for a residual discrepancy of over 20%, which is equivalent to the difference between a diploid and a tetraploid taxon. Lovis's efforts are evidence that caution must be used in comparing spore measurements made by different investigators or those made with different equipment.

Analysis of data on spore and stomate size has not usually included statistical analysis. Selling (1944) applied statistical techniques to problems of spore size, but his excellent example was not followed until recently. Analyses have seldom included simple descriptive statistics such as means and standard deviations, let alone tests of the significance of spore-size differences among related taxa.

We provide new spore and stomate measurement data for three groups of ferns in three different families in order to assess the value of cell measurements in making systematic and evolutionary inferences from herbarium specimens. The methods are not equivalent, nor are the analyses: these data sets are independently brought to bear on the general problem of cell size and fern systematics. In *Adiantum*, we document herbarium specimens of a new tetraploid taxon from eastern North America. In *Polypodium*, we explore the problem of allopolyploid versus autopolyploid origin of tetraploid *P. californicum*. In *Polystichum*, we suggest a possible candidate for a missing diploid progenitor in a new tropical montane reticulate complex.

Adiantum in Northeastern North America

Recent systematic work on the *Adiantum pedatum* complex in eastern North America has demonstrated that there are three divergent entities there: *A. pe-*

TABLE 1. Spore Size (μm) in *Adiantum pedatum*.

Taxon	Mean length (s.d.)	Observed limits	*N*	No. sporophytes
Woodland 2n	37.03 (2.494)	31.8–42.0	200	8
Serpentine 2n	42.96 (4.416)	32.0–52.8	385	16
Tetraploid	51.36 (5.162)	35.2–68.8	549	22

datum ssp. *pedatum*, a diploid of rich, deciduous woodlands; ssp. *calderi*, a diploid of serpentine substrates (formerly var. *aleuticum*; see Cody, 1983); and an unnamed tetraploid taxon of serpentine outcrops in north-central Vermont (Paris, 1986; Paris & Windham, in prep.). Although these entities have several diagnostic electrophoretic markers, they are not easily distinguished by their structural characters, most of which overlap considerably between taxa. Phenotypic plasticity further obscures species boundaries. Especially difficult to discriminate are the diploid and the tetraploid plants on serpentine, in part because the tetraploids, when growing in exposed areas, resemble their serpentine diploid progenitors quite closely. Because tetraploid plants have consistently larger spores than diploids, spore size can be used effectively to differentiate the two serpentine taxa. The predictable relationship between spore size and ploidy level in *A. pedatum* has made it possible to survey herbarium collections from serpentine areas within the Appalachian Mountain ultramafic belt in order to define the range of the new tetraploid taxon.

Materials and methods.—Spores were mounted in Hoyer's medium on glass slides and measured at 400× using an ocular micrometer. Twenty-five spores per sporophyte were measured, each in its longest dimension as it was oriented on the slide. The perispore, thin and unornamented in this group, was included in the measurement. An analysis of variance was performed on the data using the BMDP7D program (Dixon, 1983). Independence of spore size and ploidy level was tested using the G-test of Sokal and Rohlf (1981). To test whether *A. pedatum* spores swell in Hoyer's medium over time, a sample of 25 spores from each of two sporophytes was mounted in Hoyer's medium and measured after two days and again after 85 days. Significance of the difference in sample means was tested using Student's *t*-test.

Results.—Mean spore sizes for the three taxa were 37.03 μm for the woodland diploid, 42.96 μm for the serpentine diploid, and 51.36 μm for the new tetraploid (Table 1). A G-test of independence of spore size and ploidy level, using spores from cytologically or electrophoretically documented sporophytes, yielded evidence that spore size and ploidy level are not independent in this group (Table 2). Thus spore size may be used as a reliable indicator of ploidy in the *A. pedatum* complex. An analysis of variance (Table 3A) demonstrated that the means among taxa are significantly different ($P < 0.001$). Not only are both diploids different from the tetraploid, but the diploids differ significantly from one another ($P < 0.01$), as demonstrated by a GT-2 test for multiple comparisons among pairs of means (Sokal and Rohlf, 1981; Table 3B). Non-parametric tests

TABLE 2. Test of Independence of Spore Size and Ploidy for *Adiantum* Taxa on Serpentine ($N = 6$ sporophytes/taxon, 25 spores/sporophyte).

	Spores ≦ 47 μm	Spores > 47 μm	Row totals
Diploid	125	25	150
Tetraploid	50	100	150
Column totals	175	125	300
G Statistic (with Williams' correction for a 2 × 2 table) = 80.97***			

*** = $P < 0.001$.

that do not assume homoscedasticity show equally significant differences. A *t*-test of the difference in the mean size of spore samples mounted in Hoyer's medium for 2 days and for 85 days was insignificant.

POLYSTICHUM IN COSTA RICA AND ANDEAN SOUTH AMERICA

Evidence of a tropical montane polyploid complex reminiscent of well-known North Temperate complexes in the genus *Polystichum* now exists (Barrington, 1985a). The complex includes two unnamed species endemic to Costa Rica, an allotetraploid and one of its diploid progenitors. Morphologically, the best candidate for the second progenitor is *P. polyphyllum*, a common polymorphic species of the Andes, Costa Rica, and Mexico. However, *P. polyphyllum* is tetraploid based on counts from Costa Rica, Ecuador (Barrington, unpublished data), and Mexico (Smith in Barrington, 1985b). Spore length and width measurements were made to establish the relationship between spore length and ploidy level in *Polystichum* and search for a diploid cytotype of *P. polyphyllum* in northern Latin America. Included in the study set were four entities: 1) the endemic diploid from Costa Rica (ten sporophytes, documented to be diploid); 2) the

TABLE 3. Tests Comparing Mean Spore Length for *Adiantum pedatum* Taxa.

A. Analysis of variance

Source of variation	Degrees of freedom	MS	*F*
Among taxa	2	17,694.1541	857.83***
Within taxa	1131	20.6267	

B. GT-2 test for differences between means (pairwise comparisons)

Pair	Difference between means (in μm)
Woodland 2*n* vs. serpentine 2*n*	5.931**
Serpentine 2*n* vs. tetraploid	8.394**
Woodland 2*n* vs. tetraploid	14.325**

** = $P < 0.01$.
*** = $P < 0.001$.

TABLE 4. Spore Measurements (in μm) for *Polystichum* Species (30 spores/sporophyte. Standard deviation is for sample means from grand mean.)

Taxon	Spore length (s.d.)	Spore width (s.d.)	Spore volume (s.d.)	No. plants
Endemic diploid	38.52 (1.41)	28.75 (1.02)	16,940 (1590)	10
Endemic tetraploid	46.20 (3.05)	33.27 (2.50)	27,620 (5430)	10
Costa Rica *polyphyllum*	40.14 (0.59)	29.20 (0.85)	18,110 (1270)	5
Andean *polyphyllum*	41.67 (5.18)	30.25 (3.36)	20,870 (6730)	8

endemic tetraploid from Costa Rica (ten sporophytes, documented to be tetraploid); 3) *P. polyphyllum* from Costa Rica (four sporophytes, documented to be tetraploid); and 4) *P. polyphyllum* from the northern Andes from Venezuela to Ecuador (ploidy unknown).

Materials and methods.—Spore measurements of *Polystichum* species were made using a phase contrast microscope with a calibrated ocular micrometer at 400×. Measurements comprised length and width of exospore wall of those spores whose long axis was parallel to the slide surface. Damaged spores and irregular spores were excluded from measurement.

To assess the differential effects of mounting media on *Polystichum* spores over time, 20 spores from a single sporophyte (*Barrington 1275*, VT) of the endemic tetraploid were mounted in Hoyer's medium, Permount, and lactic acid on glass slides and were measured seven times over a two-week period. Spores in Hoyer's medium swelled 15% in two weeks, but spores in the other two media remained unchanged. To characterize spore size for each of the taxa in the study group, 30 spores from each sporophyte mounted in Permount were measured. Volume of each spore was computed using the formula for an ellipsoid, $V = \pi LW^2/6$ (where L is the longest dimension of the exospore and W is width measured at right angles to L). Descriptive statistics were developed using the Minitab Statistical Package (Ryan et al., 1976). To test the hypothesis that spore length could serve as a predictor of ploidy level, independence of spore size and ploidy level was tested using the G-test of Sokal and Rohlf (1981).

Results.—The mean spore lengths for the four sets of plants were 38.52 μm for the endemic diploid, 46.20 μm for the endemic tetraploid, 40.14 μm for *P. polyphyllum* in Costa Rica, and 41.67 μm for Andean *P. polyphyllum* (Table 4). The mean volumes for these same taxa were 16,940 μm^3, 22,630 μm^3, 18,110 μm^3,

TABLE 5. Test of Independence of Spore Size and Ploidy for *Polystichum* Species (N = 10 sporophytes/taxon, 30 spores/sporophyte).

	Spores ≦ 42.36 μm	Spores > 42.36 μm	Row totals
Costa Rica diploid	277	23	300
Costa Rica tetraploid	50	250	300
Column totals	327	273	600
G Statistic (with Williams' correction for a 2 × 2 table) = 393.24843***			

*** = $P < 0.001$.

and 20,870 μm^3, respectively. Spore length was a valid predictor of ploidy level for the pair of endemic Costa Rican species, since Sokal and Rohlf's G-statistic allowed the rejection of the null hypothesis that spore size and ploidy level were independent of one another (Table 5). Mean spore size of the two samples of *P. polyphyllum* plants was similar to that for the Costa Rican diploid. However, standard deviation of mean spore length by sporophyte was much more variable in Andean *P. polyphyllum* than in the first three samples, because small-spored sporophytes were encountered in Venezuela.

Polypodium in California

Polypodium glycyrrhiza is a diploid species ($n = 37$) that occurs along the west coast of North America from Monterey County, California, north to the Aleutian Islands. *Polypodium californicum* consists of two cytological races that tend to occupy different habitats. The diploid race ($n = 37$) extends from the San Francisco region south along the coast to Baja California, and the tetraploid race ($n = 74$) is found from Monterey County north to Del Norte County, California and in the foothills of the Sierra Nevada. Although there is evidence to suggest that allopolyploidy is a common mode of speciation among other *Polypodium* species in North America and Europe (Manton, 1950, 1957; Shivas, 1961; Manton & Shivas, 1953; Lloyd & Lang, 1964; Lang, 1971), the origin of the tetraploid *P. californicum* is still problematical.

Lloyd and Lang (1964) suggested that the tetraploid race arose through allopolyploidy from *P. glycyrrhiza* and diploid *P. californicum*. However, Ranker (1982) and Ranker and Mesler (1982) presented evidence based on morphology and guard-cell size that supported both allopolyploid and autopolyploid origins of the tetraploid. To test their assumption that guard cells could be used as indicators of ploidy level, guard cell data were collected from plants of known ploidy of all three taxa for this study.

Materials and methods.—Pinnae from dried specimens of known chromosome number were placed in 95% ethanol until most of the chlorophyll was extracted. The pinnae were then rehydrated in near-boiling water for about one minute and placed on a glass microscope slide with a few drops of water. Twenty-five guard cells per specimen were measured with an ocular micrometer at a magnification of 400×.

Results.—Inspection of the data from diploid *P. californicum* revealed a strongly bimodal distribution of guard-cell size: a cluster of four individuals with smaller guard cells from drier, more southern sites and a cluster of three individuals with larger guard cells from more mesic, northern sites (Table 6). These clusters were treated as distinct groups in subsequent analyses. The mean guard-cell sizes were 50.4 μm for *P. glycyrrhiza*, 54.3 μm for diploid *P. californicum* from southern California, 65.2 μm for diploid *P. californicum* from northern California, and 60.4 μm for tetraploid *P. californicum* (Table 6). A nested analysis of variance showed that there were significant amounts of variation both among plants within taxa and among taxa (Table 6). Pairwise comparisons of means using the GT-2 method indicated that all means differed significantly ($P < 0.05$).

TABLE 6. Comparison of Mean Guard-Cell Length for *Polypodium* Taxa.

A. Guard-cell measurements (N = 25 guard cells/plant)

Taxon	Mean length, μm	s.d.	No. of plants	Range of plant means
P. glycyrrhiza, 2n	50.4	3.293	22	42.8–57.8
P. californicum, 2n				
Southern cluster	54.3	1.352	4	52.7–55.6
Northern cluster	65.2	3.007	3	62.1–68.1
P. californicum, 4n	60.4	3.564	12	54.5–66.9

B. Nested analysis of variance for guard-cell data

Source of variation	Degrees of freedom	MS	F
Among taxa	3	1468.7033	36.45***
Among plants within taxa	37	40.2949	32.53***
Within plants	984	1.2389	

*** = $P < .001$.

ANALYSIS AND DISCUSSION

Measurements of spores and stomates serve as useful probes for establishing hypotheses of evolutionary relationships within polyploid complexes. Spore and stomate size of polyploids is dependent on two factors: size of cells in diploid progenitors and ploidy level. Taken together, these two factors may be used to predict cell size of missing members of polyploid complexes from cell-size means of the known members, so long as environmental variation does not compromise the analysis.

In this paper we have provided new evidence that cell size is correlated with ploidy level in ferns from investigations of three reticulate complexes in three different families. That each study involves a tetraploid and at least one of its progenitors is suggested by results of other studies, cytological or electrophoretic.

Little information exists about the variation in size of spores or stomates in the diploid members of a polyploid complex. The two eastern North American diploid taxa in the *Adiantum pedatum* complex differ significantly with respect to spore size. In this case, the observed differences in size are consistent with the theory that species in insular habitats have larger spores (Carlquist, 1966). The serpentine taxon of *A. pedatum* has larger spores than its ally, which is widespread in woodlands. We suggest that serpentine outcrops are analogous to islands for serpentine-adapted plant species, so that spores of serpentine *Adiantum pedatum* have responded to selection for limited dispersability.

We contend that the spore size of an allotetraploid can be predicted from the known size of its diploid progenitors by multiplying a constant reflecting the increase (in volume or linear dimension) due to polyploidy by the mean of the two diploid spore volumes. Increase in cell size due to polyploidy is not necessarily consistent among species or even among tissues of a single organism (Sin-

nott, 1960). However, the assumption that doubling of chromosome number yields a doubling of cell volume gave meaningful results in this study. Using a sphere as a model, doubling volume would give a concomitant increase of 1.26 diameters. We multiplied the average of the mean lengths of the tetrahedral-globose spores of two diploid adiantums by 1.26 to predict the mean spore length for the tetraploid *Adiantum* from Vermont to be 50.39 μm, which is close to the observed mean spore length of 51.36 μm.

We predicted the mean spore volume of a second progenitor for the tetraploid *Polystichum* endemic to Costa Rica to be 10,670 μm^3 by solving for a missing diploid rather than for the tetraploid. Although *P. polyphyllum* is tetraploid in Costa Rica and thus could not serve as a progenitor of the endemic tetraploid, a diploid progenitor of *P. polyphyllum* could be an ancestor. The sole diploid progenitor of an autotetraploid *P. polyphyllum* would be predicted to have a spore volume of 9050 μm^3. Although spores of most Andean *P. polyphyllum* measured for this study were within the range for a tetraploid, a few collections from Venezuela and Colombia had smaller spores that approached the predicted size for diploids. Hence, the data suggest that a diploid progenitor of *P. polyphyllum* and the endemic Costa Rican tetraploid may be found in the northern-most Andes.

Guard-cell size can also be used as a valid indicator of ploidy. The results from *Polypodium* show that in northern California, where the diploid *P. glycyrrhiza* is sympatric with tetraploid *P. californicum*, the two can be distinguished using guard-cell size. South of this region, the relatively small guard cells of diploid *P. californicum* make this character a less useful predictor. These data also can be used to address the question of the origin of the tetraploid cytotype of *P. californicum*. They indicate that this taxon could have arisen through allopolyploidy from two progenitors, one similar to the southern cluster of diploid *P. californicum* and the other *P. glycyrrhiza*. Both the southern and northern clusters of diploid *P. californicum* have guard cells that are too large to accommodate an autopolyploid origin of the tetraploid. Environmentally induced variability similar to that encountered by Cox and Hickey (1984) may complicate the interpretation of stomate-size data in this group.

Calculation of expected guard-cell lengths for the triploid and tetraploid cytotypes of the hybrid between *P. acrostichoides* and *P. braunii* in Vermont provides another test of the contention that cell sizes of unknown members of polyploid complexes can be predicted from cell sizes of known members. Data from Barrington (1986) for guard-cell lengths of the four taxa involved, along with values predicted from guard-cell lengths of the progenitors and from ploidy level, are reported in Table 7 (see Appendix). The predicted values for the hybrid guard cells are similar to the observed values. These calculations demonstrate that the unexpected values for hybrid guard-cell sizes are predicted from a model including two factors, one reflecting ploidy level and the other the size of the progenitor guard cells. The close match of the observed value to the predicted value for the triploid suggests that a two-fold increase in the volume of polyploidized fern cells may be common enough to use as a preliminary indicator of ploidy level in ferns.

TABLE 7. Analysis of Guard-Cell Pair Measurements in μm for *Polystichum* ×*potteri* and its progenitors (N = 30 guard cell pairs per sporophyte).

Taxon	Ploidy level	Genomic constitution	Observed mean guard-cell length (s.d.)	Predicted mean guard-cell length
acrostichoides	2n	aa	53.6 (0.97)	—
braunii	4n	bbbb	47.8 (1.14)	—
×*potteri*	3n	abb	50.0 (1.83)	49.7
×*potteri*	4n	aabb	59.8, 64.0*	58.5

* Two counts of same sporophyte, collections from GH and MICH, respectively.

In summary, using data from three genera we have provided additional support for the hypothesis that ploidy level and cell size are correlated in ferns. We have also provided some tests of the hypothesis that cell sizes of diploids and their tetraploid derivatives can serve as a basis for predicting the size of missing members of their reticulate complex. We suggest that more exhaustive data sets from better-known reticulate complexes would be very enlightening tests of this simple hypothesis. Especially important would be a critical analysis of complexes in which polyploids fail to conform to predictions, such as Appalachian *Dryopteris* (Wagner, 1971).

LITERATURE CITED

BARRINGTON, D. S. 1985a. Hybridisation in Costa Rican *Polystichum*. Proc. Roy. Soc. Edinburgh 86B:335–340.

———. 1985b. The present evolutionary and taxonomic status of the fern genus *Polystichum*: The 1984 Botanical Society of America Pteridophyte Section Symposium. Amer. Fern J. 75: 22–28.

———. 1986. The morphology and cytology of *Polystichum* × *potteri* hybr. nov. (= *P. acrostichoides* × *P. braunii*). Rhodora 88:297–313.

BLASDELL, R. F. 1963. A monographic study of the fern genus *Cystopteris*. Mem. Torrey Bot. Club 21:1–102.

BRITTON, D. M. 1968. The spores of four species of spinulose wood ferns (*Dryopteris*) in Eastern North America. Rhodora 70:340–347.

BROWN, D. F. M. 1964. A monographic study of the fern genus *Woodsia*. Beih. Nova Hedwigia 16: 1–154.

BUTTERS, F. K. and R. M. TRYON Jr. 1948. A fertile mutant of a *Woodsia* hybrid. Amer. J. Bot. 35: 132.

CARLQUIST, S. 1966. The biota of long-distance dispersal. III. Loss of dispersibility in the Hawaiian flora. Brittonia 18:310–335.

CODY, W. J. 1983. *Adiantum pedatum* ssp. *calderi*, a new subspecies in northeastern North America. Rhodora 85:93–96.

COX, P. A. and R. J. HICKEY. 1984. Convergent megaspore evolution and *Isoetes*. Amer. Naturalist 124:437–441.

DIXON, W. J. (ed.) 1983. *BMDP statistical software*. Berkeley: Univ. California Press.

HAGENAH, D. J. 1961. Spore studies in the genus *Cystopteris* I. The distribution of *Cystopteris* with non-spiny spores in North America. Rhodora 63:181–193.

KOTT, L. and D. M. BRITTON. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. Canad. J. Bot. 61:3140–3163.

LANG, F. A. 1971. The *Polypodium vulgare* complex in the Pacific Northwest. Madroño 21:235–254.
LAWTON, E. 1932. Regeneration and induced polyploidy in ferns. Amer. J. Bot. 19:303–334.
LLOYD, R. M. and F. A. LANG. 1964. The *Polypodium vulgare* complex in North America. Brit. Fern Gaz. 9:168–177.
LOVIS, J. D. 1964. The taxonomy of *Asplenium trichomanes* in Europe. Brit. Fern Gaz. 9:147–160.
———. 1977. Evolutionary patterns and processes in ferns. Advances Bot. Res. 4:229–415.
——— and T. REICHSTEIN. 1968. Die zwei diploiden *Asplenium trichomanes* × *viride*-Bastarde und ihre Fähigkeit zur spontanen Chromosomenverdoppelung. Bauhinia 4:53–63.
MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge Univ. Press.
———. 1957. The problem of *Polypodium virginianum*. Amer. Fern J. 47:129–134.
——— and M. SHIVAS. 1953. Two cytological forms of *Polypodium virginianum* in eastern North America. Nature 172:410–411.
PARIS, C. A. 1986. A biosystematic investigation of the *Adiantum pedatum* complex in eastern North America. M.S. Thesis, University of Vermont, Burlington.
RANKER, T. A. 1982. The *Polypodium vulgare* complex in Northern California. M.A. Thesis, Humboldt State University, Arcata, California.
——— and M. R. MESLER. 1982. The origin of tetraploidy in *Polypodium californicum*: an autopolyploid hypothesis. Bot. Soc. America, Misc. Publ. 162:77 (abstract).
RYAN, T. A. Jr., B. L. JOINER, and B. F. RYAN. 1976. *Minitab student handbook*. Boston: PWS Publishers.
SCHNELLER, J. 1974. Untersuchungen an einheimischen Farnen, insbesondere der *Dryopteris filix-mas*-Gruppe. 1 Teil. Ber. Schweiz. Bot. Ges. 84:195–217.
SELLING, O. H. 1944. Studies in the recent and fossil species of *Schizaea*, with particular reference to their spore characters. Acta Horti Gothob. 16:1–112.
SHIVAS, M. G. 1961. Contributions to the cytology and taxonomy of species of *Polypodium* in Europe and America. II. Taxonomy. J. Linn. Soc., Bot. 58:27–38.
SINNOTT, E. W. 1960. *Plant morphogenesis*. New York: McGraw-Hill.
SOKAL, R. R. and F. J. ROHLF. 1981. *Biometry*. San Francisco: W. H. Freeman & Co.
STEBBINS, G. L. 1950. *Variation and evolution in plants*. New York: Columbia Univ. Press.
WAGNER, W. H. Jr. 1954. Reticulate evolution in the Appalachian aspleniums. Evolution 8:103–118.
———. 1966. New data on North American oak ferns, *Gymnocarpium*. Rhodora 68:121–138.
———. 1971. Evolution of *Dryopteris* in relation to the Appalachians. Pp. 147–191 in *The distributional history of the biota of the southern Appalachians. Part II. Flora*, ed. P. C. Holt. Research Division Monograph 2. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.
WINDHAM, M. D. and C. H. HAUFLER. 1986. Biosystematic uses of fern gametophytes derived from herbarium specimens. Amer. Fern J. 76:114–128.

APPENDIX

To calculate the predicted length values for the hybrids, haploid length values for the progenitor genomes were derived by multiplying the observed guard-cell lengths by 1/1.26 (for the diploid species) and $1/1.26^2$ (for the tetraploid species). 1/1.26 is the ratio of diameters of two spheres, the first having half the volume of the second. Then the predicted value was computed by multiplying the average of the haploid values for each component genome—(a + a + b + b)/4 for the tetraploid cytotype and (a + b + b)/3 for the triploid cytotype—by a coefficient reflecting increase in diameter expected for a given ploidy level of the hybrid ($1.26^{3/2}$ for the triploid, 1.26^2 for the tetraploid).

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

AMERICAN FERN JOURNAL

Volume 76

Number 4

October–December 1986

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

The Neotropical Fern Genus *Olfersia* *Robbin C. Moran* **161**

***Trichomanes* in Florida** *Clifton E. Nauman* **179**

Some New Names and Combinations in Pteridaceae *Rolla Tryon* **184**

A Novel Method for Surface-sterilizing and Sowing Fern Spores *Thomas R. Warne, Gary L. Walker,* and *Leslie G. Hickok* **187**

Shorter Note

***Osmunda cinnamomea* forma *frondosa* in the Coastal Plain of Georgia and Florida** *Richard Carter* and *Wayne R. Faircloth* **189**

Review **190**

Referees, 1986 **190**

Index to Volume 76—1986 **191**

Information for Authors **Cover 3**

The American Fern Society

Council for 1986

FLORENCE S. WAGNER, Dept. of Botany, University of Michigan, Ann Arbor, MI 48109. *President*

JUDITH E. SKOG, Biology Dept., George Mason University, Fairfax, VA 22030. *Vice-President*

W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233. *Secretary*

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916. *Treasurer*

DAVID S. BARRINGTON, Dept. of Botany, University of Vermont, Burlington, VT 05405. *Records Treasurer*

JAMES D. MONTGOMERY, Ecology III, R.D. 1, Berwick, PA 18603. *Back Issues Curator*

ALAN R. SMITH, Dept. of Botany, University of California, Berkeley, CA 94720. *Journal Editor*

DAVID B. LELLINGER, Smithsonian Institution, Washington, DC 20560. *Memoir Editor*

DENNIS Wm. STEVENSON, Dept. of Biological Sciences, Barnard College, Columbia University, New York, NY 10027. *Fiddlehead Forum Editor*

American Fern Journal

EDITOR

ALAN R. SMITH . . . . . Dept. of Botany, University of California, Berkeley, CA 94720

ASSOCIATE EDITORS

GERALD J. GASTONY . . . . Dept. of Biology, Indiana University, Bloomington, IN 47401

CHRISTOPHER HAUFLER . . . . . Dept. of Botany, University of Kansas, Lawrence, KS 66045

DAVID B. LELLINGER . . . . . U.S. Nat'l Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560

TERRY R. WEBSTER . Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at the Pringle Herbarium, University of Vermont, Burlington, VT 05405. Second-class postage paid at Burlington, VT, and additional entry point.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, R.D. 1, Berwick, PA 18603.

Changes of address, dues, and applications for membership should be sent to the Records Treasurer.

General inquiries concerning ferns should be addressed to the Secretary.

Subscriptions $12.00 gross, $11.50 net if paid through an agency (agency fee $0.50); sent free to members of the American Fern Society (annual dues, $10.00 + $4.00 mailing surcharge beyond U.S.A., Canada, and Mexico; life membership, $200.00).

Back volumes 1910–1978 $5.00 to $6.25 each; single back numbers of 64 pages or less, $1.25; 65–80 pages, $2.00 each; over 80 pages, $2.50 each, plus shipping. Back volumes 1979 et seq. $8.00 each; single back numbers $2.00 each, plus shipping. Ten percent discount on orders of six volumes or more.

POSTMASTER: Send address changes to AMERICAN FERN JOURNAL, Dept. of Botany, University of Vermont, Burlington, VT 05405.

Fiddlehead Forum

The editors (Dennis Wm. and Jan Wassmer Stevenson) welcome contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

Spore Exchange

Mr. Neill D. Hall, 1230 Northeast 88th Street, Seattle, WA 98115, is Director. Spores exchanged and lists of available spores sent on request.

Gifts and Bequests

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Botanical books, back issues of the Journal, and cash or other gifts are always welcomed, and are tax-deductible. Inquiries should be addressed to the Secretary.

American Fern Journal 76(4):161–178 (1986)

The Neotropical Fern Genus *Olfersia*

ROBBIN C. MORAN
Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, Illinois 61820

Olfersia Raddi (Dryopteridaceae) consists of a single species, *O. cervina* (L.) Kunze, which is widely distributed in forests of the neotropics. This species differs from all other dryopteroid ferns by its numerous, fine, parallel veins that connect at their tips by a submarginal vein. Other features that distinguish *Olfersia* from many dryopteroid ferns are its strongly differentiated sterile and fertile leaves, simply pinnate sterile lamina, entire pinnae, and conform apical pinna. *Olfersia* is morphologically most similar and cladistically most closely related to *Polybotrya*, a genus that also has strong sterile-fertile leaf dimorphism. Most recent pteridologists have placed *Olfersia* in *Polybotrya*; however, *Olfersia* differs by the characters noted above and lacks the unique stem anatomy of *Polybotrya*.

This paper, an outgrowth of my work on *Polybotrya* (Moran, 1986), is based on a study of about 550 herbarium sheets that represent approximately 335 individual collections from 29 herbaria (see Acknowledgments). I spent seven months collecting *Olfersia* and *Polybotrya* in Costa Rica, Ecuador, Peru, and Venezuela and was able to study living plants of *Olfersia* in each of those countries. Important observations were made about the biology of *Olfersia* that would have been impossible to discern from herbarium specimens, such as the duration of fertile leaves on the stem, the orientation of sterile vs. fertile leaves, and how often the plants were terrestrial or became scandent. Herbarium, cytological, and anatomical material were also collected during field work.

TAXONOMIC HISTORY

Olfersia cervina has received various treatments from pteridologists. It was first described by Linnaeus (1753), who placed it in *Osmunda*, at that time a polyphyletic genus. His specific epithet *cervina* means "deer," and he may have been alluding to the fancied resemblance of the velvet on a deer's antlers to the brown sporangia that cover the fertile pinnules of *Olfersia*. Linnaeus never saw a specimen of this species, but knew of its existence from the excellent engravings of Plumier (1705) and Petiver (1712). Swartz (1806) placed this species in *Acrostichum* because he recognized that the sporangial capsules have a vertical, bowlike annulus (his group "Gyratae"), unlike true *Osmunda*, which has only a lateral patch on one side of the capsule wall (his group "Spurie Gyratae"). Swartz's genus *Acrostichum*, consisting of all ferns having the sori spread across the abaxial surface of the leaf and lacking an indusium, was also a large, polyphyletic group.

Raddi (1819) described the genus *Olfersia*, honoring Dr. Ignaz Fraz Werner von Olfers (1793–1871), a botany professor from Rio de Janeiro whom Raddi probably met during his travels with the Austrian Expedition to Brazil (1817–

MISSOURI BOTANICAL

FEB 5 1987

1818). While in Rio de Janeiro, Raddi collected the type species of his new genus, *O. corcovadensis*, at Mt. Corcovado. But Raddi never compared *Olfersia* with the closely related genus *Polybotrya* or with *A. cervinum*, suggesting that he was unaware of their close relationship. He distinguished *Olfersia* from other fern genera by its fertile pinnae that are entire and have sporangia on both surfaces.

Kaulfuss (1824), apparently unaware of *Olfersia corcovadensis*, placed *A. cervinum* in *Polybotrya*, a genus described by Willdenow (1810). He made this decision because *Polybotrya* was said to have sporangia that cover both surfaces of the pinnules—the condition that was thought to occur in *A. cervinum*. Based on this criterion, Sprengel (1827) put *O. corcovadensis* in *Polybotrya*, alongside *A. cervinum*. Apparently, prior to Sprengel's work it was not realized how closely these two plants were related. Link (1833) also thought that *O. corcovadensis* should be placed in *Polybotrya*, but provided the name *P. raddiana*—a superfluous name for *P. corcovadensis* (Raddi) Sprengel. Presl (1851) carefully studied the fertile leaves of *O. cervina* and concluded that, unlike *O. corcovadensis*, the sporangia do not occupy both surfaces of the fertile pinnules. Thus, he created the genus *Dorcapteris* to accommodate *O. cervina*. Unfortunately, he included in *Olfersia* many unrelated species of *Elaphoglossum* (Lomariopsidaceae) and *Stenochlaena* (Blechnaceae).

Throughout the first half of the 1800s, pteridologists maintained *cervina* and *corcovadensis* as distinct. Finally, Hooker (1864) observed that intermediates exist in the shape of the fertile pinnules, the main character that had been used to separate the two plants. Accordingly, he placed the two plants in synonymy, using the name *A. cervinum*. Although later pteridologists have agreed that *cervina* and *corcovadensis* are the same species, they have differed in their generic placements. Most have followed Christensen (1905), who placed *O. cervina* in *Polybotrya*, but Smith (1875), Brade (1971), and Pichi Sermolli (1977) have maintained *Olfersia* as a distinct genus.

Geography

Compared with most other dryopteroid ferns, *Olfersia* has an extensive range. *Polybotrya*, for example, with its 35 species, is essentially coextensive with *O. cervina*—both occur from Oaxaca/Veracruz, Mexico, southward through Central America, from northern South America to Bolivia and Rio Grande do Sul, Brazil, and from Cuba to Grenada in the West Indies (Fig. 1; Moran, 1986). *Olfersia* inhabits wet, shaded, tropical forests from steamy rain forests at sea level to cool cloud forests up to 2000 m. It is most common in primary forests and rarely occurs in disturbed habitats. Its distribution correlates generally with the moist montane regions of tropical America. Few collections have been made in areas of less topographic relief, such as the Matto Grosso, the Brazilian Amazon, and the Guyanan Highlands.

Olfersia occurs on only one oceanic island—Cocos Island in the Pacific Ocean, about 500 kilometers (310 miles) southwest of Costa Rica. This finding supports Tryon's (1970) contention that the ferns of oceanic islands tend to be those that

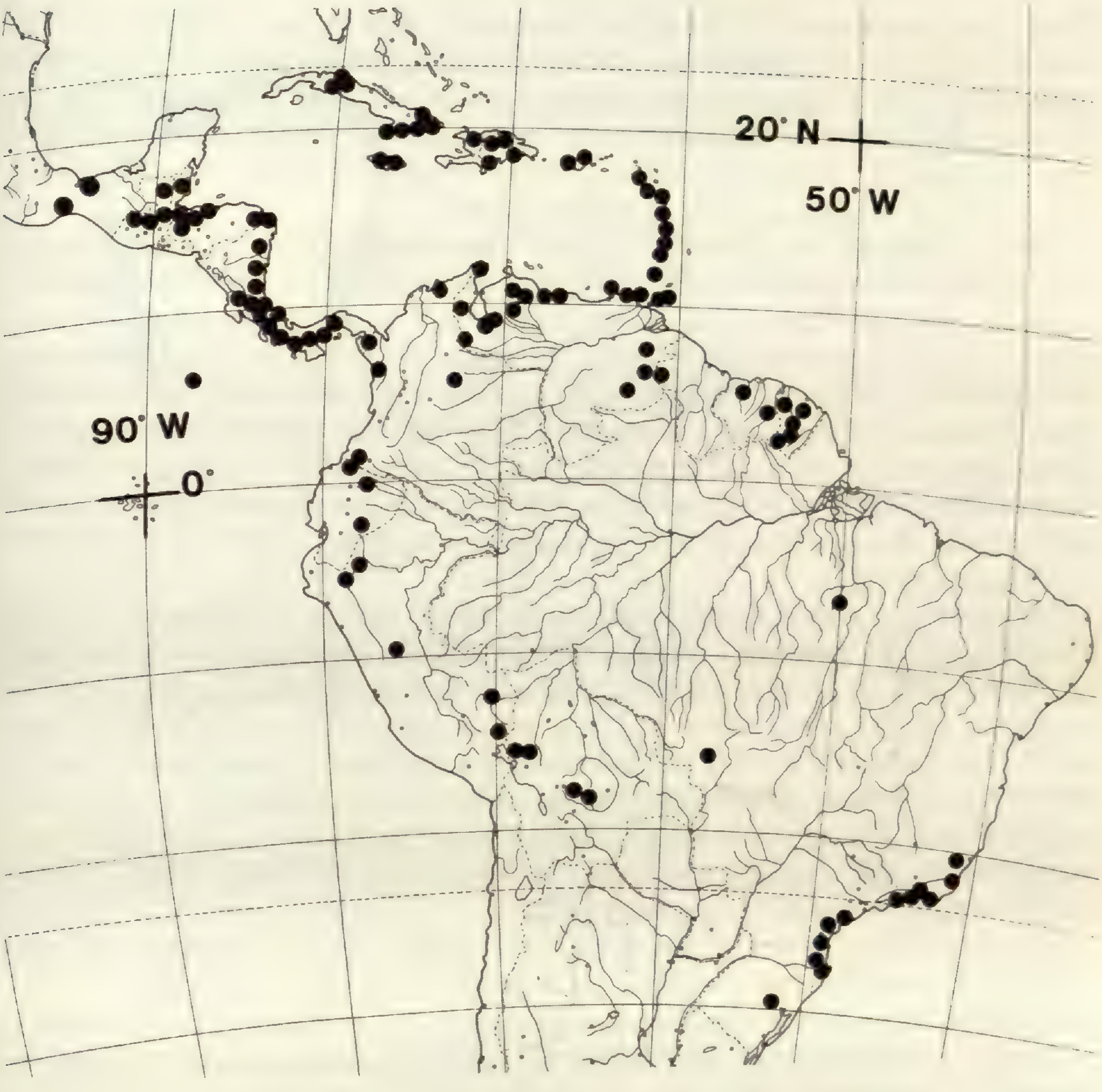


FIG. 1. Geographic distribution of *Olfersia cervina*.

are widespread beyond the source area for the island (the source area for Cocos Island is defined by Tryon as Central America and Colombia). Widely distributed species have a broader ecological amplitude and therefore establish themselves more often on oceanic islands because of their ability to grow under a wide range of environments (Tryon, 1970).

COMPARATIVE MORPHOLOGY AND ANATOMY

Stem.—The stem of *Olfersia* is short-creeping with internodes generally 1–3 cm apart. Although *Olfersia* is frequently terrestrial, it has a tendency to grow on logs and to climb up tree trunks for distances up to 1 m. Since its stem is of limited growth, it never becomes high-climbing like most species of *Polybotrya*. Consequently, it makes an excellent pot plant (Walker, 1985a).

Stem anatomy is the most important difference between *Olfersia* and *Poly-*

botrya. The meristeles of *Olfersia* are not readily seen in cross section because each meristele is surrounded by whitish, parenchymatous cells of the ground tissue and not by a sheath of dark sclerenchyma fibers as in *Polybotrya* (Fig. 2a, b). The arrangement of meristeles around the center of the stem is irregular, not even and circular as in *Polybotrya*. The meristeles also have irregular shapes that vary from round to oblong to slightly lobed.

The epidermis and hypodermis consist of dark, thick-walled fibers that in cross section give the appearance of a black ring encircling the stem. This ring contrasts sharply with the whitish parenchyma of the underlying ground tissue, which contains numerous amyloplasts. Embedded in the ground tissue are myriad clusters of brachysclereids, each having dark cell walls with numerous pits. These darkened clusters also contrast sharply with the whitish ground parenchyma and are perhaps the most conspicuous feature of a freshly cut stem. Internal glandular hairs such as those that occur in the ground tissue of stems and petioles of many dryopteroid ferns (Mehra & Mittal, 1961) are apparently lacking in *Olfersia*.

Stem scales.—Golden or yellowish scales, which usually darken upon drying, densely cover the stems of *Olfersia*. They lie slightly appressed to the stem and are linear with an attenuate tip. The abruptly truncated base is attached along a narrow, slightly curved line, not at a single point. The scales are thin and membranaceous, and the cell outlines are easily visible. The margins are usually denticulate, although some scales appear entire, except for a few minute teeth near the apex. The teeth are always composed of the projecting end walls from two adjacent cells (Fig. 2e). The scales of *Olfersia* are similar to those of *Polybotrya osmundacea* and *P. polybotryoides*.

Roots.—*Olfersia* produces numerous, dark, tough, fibrous roots from all sides of the stem; these bear no positional relationship to the leaf bases. Anatomically, the roots of *Olfersia* are like those described for *Polybotrya* by Moran (1986).

Petiole.—A cross section of an *Olfersia* petiole reveals vascular bundles arranged in a mushroom-like shape, with the base of the mushroom oriented adaxially (Fig. 3b). A similar pattern also occurs in *Cyclodium trianae* (pers. obs.), *Polybotrya* (Moran, 1986), *Maxonia* (Walker, 1972), and in some species of Lomariopsidaceae (Holttum, 1978). Further study of petiole anatomy in dryopteroid genera may provide helpful information for assessing relationships. Scales occur on the petiole bases of *Olfersia*. Although similar to those on the stem, these scales are usually wider and shorter.

The petiole base in some ferns is specialized to accumulate food and to persist as a storage organ long after the rest of the leaf to which it was attached has withered and decayed. These specialized petiole bases, called "trophopods," are a new source of systematic data in ferns (Wagner & Johnson, 1983). *Olfersia*, however, lacks trophopods, and the food-storing function is performed by the stem, which contains abundant starch grains in the cells of its ground parenchyma.

Rhachis and costae.—In fresh material, the rhachis and costae of *Olfersia* are rounded or shallowly grooved adaxially. Two linear, yellowish, aerophores occur on the sides of the rhachis and petiole. When dry, the large, parenchymatous

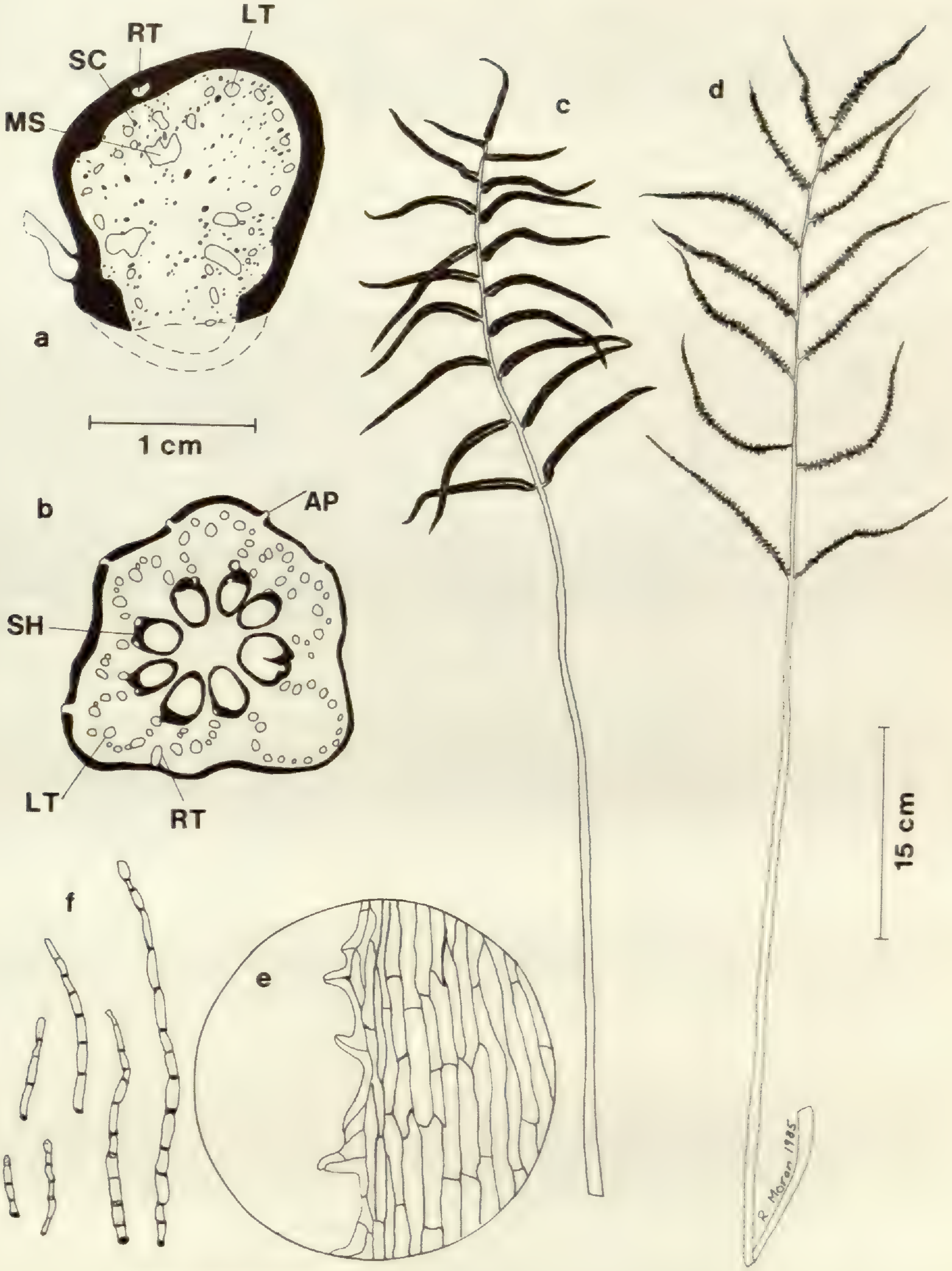


FIG. 2. Various features of *Olfersia cervina*. a, cross section of the stem; b, cross section of a *Polybotrya* stem for comparison; c, fertile leaf with entire pinnae; d, fertile leaf with pectinate pinnae; e, close-up of stem scale margin; f, reduced, uniseriate scales from the costa and laminar tissue. a, d, e, f: *Moran 2181* (F). b: *Moran 2182* (F). c: *Brackenridge s.n.*, U.S. Exploring Expedition (PH). AP, aerophore; LT, leaf trace; MS, meristele; RT, root trace; SC, sclereid cluster; SH, sclerenchyma sheath surrounding meristele.

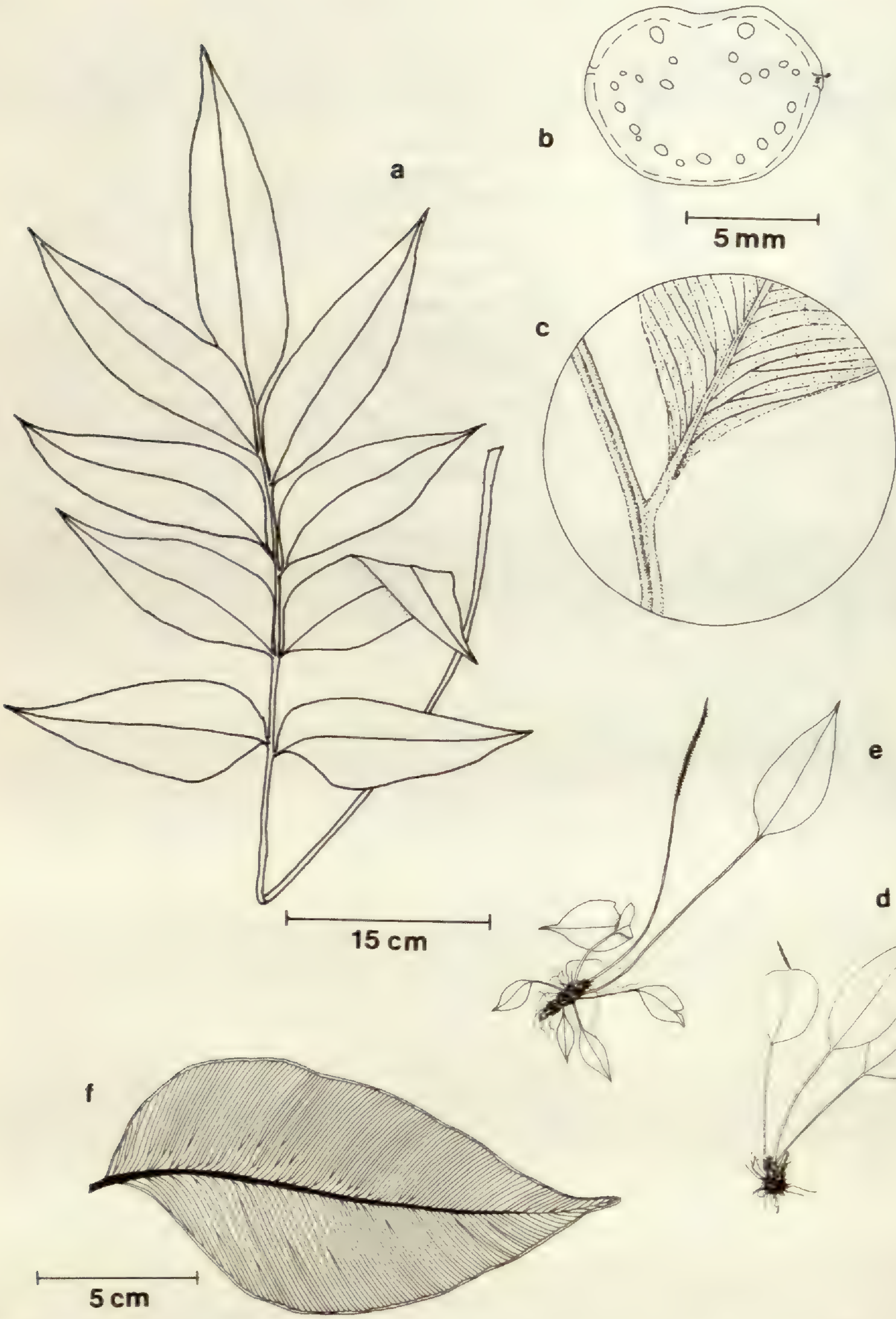


FIG. 3. Sterile leaves of *Olfersia cervina*. a, mature fertile leaf; b, cross section of petiole base showing vascular bundles arranged in a mushroom shape; c, close-up of rhachis-pinnae jucture; d, e: juvenile plants with precociously fertile leaves; f, pinnae showing venation, note submarginal connecting strand. a, b, c, f: *Moran 2181* (F). e, d: *Antonio 3715* (MO).

cells in the interior of the rhachis collapse, indenting the adaxial surface of the rhachis. The two aerophores on each side of the rhachis and petiole (Fig. 3b) also collapse, since they are composed of soft walled, parenchymatous cells. As a result, the rhachis and costae become trisulcate. The costae, when dry, may remain rounded or become shallowly grooved. These grooves, however, fade out before they reach the rhachis so that the costal grooves are not continuous with the rhachis grooves. Since in other genera of dryopteroid ferns the grooves are decurrent into one another (Holttum, 1984), the non-decurrent grooves of *Olfersia* are interpreted as derived.

Leaf apex.—*Olfersia* has a conform apex similar to the lateral pinnae (Figs. 2c, d, 3a). *Polybotrya,* however, has a pinnatifid apex even in its simply pinnate species. Two species of *Polybotrya, P. espiritosantensis* and *P. polybotryoides,* have subconform apices, but these species are clearly derived within the genus and do not indicate a relationship with *Olfersia* (Moran, 1986). The leaf apex, therefore, affords a convenient distinguishing trait between the two genera.

Venation.—*Olfersia* has numerous, long, fine, parallel veins connected at their tips by a submarginal strand (Fig. 3f). The veins usually branch once or twice near the costa and then continue unbranched to the connecting strand. All the veins are approximately the same width and do not form distinctly pinnate groups. The only species of *Polybotrya* having venation remotely similar to that of *Olfersia* are *P. fractiserialis* and *P. sorbifolia,* but they lack a submarginal connecting strand, have more branched veins, and form distinctly pinnate groups. The venation of *Olfersia* is not only unlike that of *Polybotrya* but unlike that of any dryopteroid genus. This dissimilarity emphasizes the distinctness of *Olfersia.*

Hairs.—The leaf of *Olfersia* is usually described as glabrous; however, the abaxial surface is sparsely beset with inconspicuous, slender "hairs" 0.1–1.2 mm long (Fig. 2f). Usually seen on young, incompletely expanded leaves and along the costa of mature leaves where the lamina has been protected from abrasion, these tiny, uniseriate structures are "hairs" by definition only; developmentally they are scales. Evidence for their scalelike nature comes from comparison with such other dryopteroid genera as *Polybotrya, Dryopteris,* and *Arachniodes,* in which the "hairs" intergrade with scales. In these genera, the "hairs" at the apex of the costa are tiny, less than 0.1 mm long, two to fifteen celled, mostly appressed to the surface, and have reddish cross walls. Each cell is slightly flattened and the apical cell of the hair is conform, unlike the true hairs of dryopteroid ferns. Towards the middle of the costa these hairs elongate by increasing the number of cells, and some become two to three seriate near the base, that is, they become a scale. On the rhachis and base of the costa, the scales become large and polyseriate. Every intermediate form can be found; hence the evidence that the hairs are actually reduced scales. I suggest that these tiny, uniseriate scales be called "proscales."

Like most species of *Dryopteris* (Christensen, 1913), *Olfersia* lacks true hairs. True hairs in dryopteroid ferns do not intergrade with scales and are erect or spreading—never appressed. True hairs are whitish, cylindrical, and thicker walled than the proscales. Their apical cells are usually of a different shape than the rest of the cells, often being acicular or acute (Moran, 1986, fig. 10).

Fertile leaves.—*Olfersia* produces strongly differentiated sterile and fertile leaves. The fertile leaves are like a skeleton of the sterile leaves because the green, photosynthetic tissue has been reduced to the axes as a narrow wing. Such extreme dimorphy is termed "holodimorphy" (Wagner & Wagner, 1977) and involves many characters, not merely the reduction of the green lamina. In *Olfersia*, the sterile and fertile leaves also differ in size, dissection, orientation, venation, and duration on the stem. Since *Olfersia* has the same syndrome of foliar dimorphy characters as that described for *Polybotrya* by Moran (1986), only the most conspicuous differences will be mentioned.

The sterile and fertile leaves differ in size, with the laminae of the fertile leaves smaller than those of the sterile. The total length of the fertile leaves, however, is greater than that of the sterile because of the elongated petiole, which is generally twice the length of the lamina. The two terrestrial species of *Polybotrya* are like *Olfersia* in having taller fertile leaves. The climbing species of *Polybotrya*, however, have fertile leaves that are shorter than the sterile leaves. Presumably, taller fertile leaves are advantageous for terrestrial plants since the sporangia are borne at a higher position where drying occurs more readily and the spores are more likely to be picked up by air currents before they hit the ground.

The orientation of sterile and fertile leaves also differs. Sterile leaves, which do most of the photosynthesis, recline with age, orienting themselves perpendicularly to the sun's rays. This position would be advantageous in a shaded, tropical forest where sunlight is reduced. Fertile leaves remain erect, however, which presumably promotes drying of sporangia. Differences in the orientation of sterile and fertile leaves typically occur in ferns that exhibit dimorphy (Wagner & Wagner, 1977). This correlation suggests that strong selective pressures exist for differences in sterile-fertile leaf orientation. After shedding their spores, fertile leaves quickly wither. Rapid senescence probably contributes to the plant's economy since large quantities of carbohydrates and mineral nutrients are not spent to maintain a structure that lacks appreciable photosynthetic surface. As a corollary to their ephemeral character, the fertile leaves are of "cheaper" construction, that is, they have little of the rigid support collenchyma found in sterile leaves.

Seasonal production of fertile leaves accompanies sterile-fertile leaf dimorphy in some ferns, but seasonality does not appear to be present in *Olfersia*. On the Atlantic coastal plain of Costa Rica, I saw populations of *Olfersia* that contained plants in various stages of fertile leaf production; some plants had fertile leaves in the crozier stage, others had fertile leaves in the spore-releasing stage, and in some individuals the fertile leaves had died and withered. This observation suggests continuous rather than seasonal production. Dr. Henk van der Werff, working in the state of Falcón, Venezuela, also found that *Olfersia* produced fertile leaves throughout the year (pers. comm.). The question of seasonality in fertile leaves, however, needs further study.

Olfersia produces fertile leaves freely on both terrestrial and climbing stems. In contrast, the climbing species of *Polybotrya* almost always produce fertile leaves on the scandent portion of the stem. Production of fertile leaves on the

terrestrial stem of a climbing *Polybotrya* is extremely rare; I know of only three examples among the thousands of plants I have seen in the field and in herbaria. This variability in the site of fertile leaf production in *Olfersia*, compared to its inflexibility in *Polybotrya*, is an important behavioral character differentiating the two genera.

Fertile pinnae.—Two extreme forms of fertile pinnae occur in *Olfersia*. The first type is pectinate (Fig. 2d), and sporangia are produced only on the abaxial surface of the leaf. When dry, however, the adaxial surface retroflexes so that sporangia appear to occupy all sides of a now cylindrical pinnule. The second type of pinnae is entire and has sporangia on both surfaces (Fig. 2c; amphiacrostichoid). The pectinate type prevails throughout most of the range, but the amphiacrostichoid type is common in southeastern Brazil, where it accounts for approximately 80% of the specimens. Although the two types of pinnae differ greatly in appearance, they may occur on the same plant and therefore only represent forms. Accordingly, the amphiacrostichoid type, which has been named *O. corcovadensis*, is placed in synonymy.

Diplodesmic veins.—The sorus of *Olfersia* contains a special vascular supply that lies just beneath the sporangia but above the normal veins, so that a cross section of the sorus reveals two sets of veins at two levels (Fig. 4c, d). This condition, termed "diplodesmic," is also found in *Polybotrya*. Diplodesmic veins, in contrast to the normal veins, are very delicate, obscure, and abundantly anastomosed. The extensive anastomoses connect infrequently with the normal veins, which are usually thick, prominent, and rarely branched. Diplodesmic veins are apparently homologous with the veins of the sterile leaf. This homology can be seen in transitional (part sterile, part fertile leaves) where the tips of the sterile veins grade into the diplodesmic veins in the fertile parts (Fig. 4c, d). The leaves need to be cleared to see this gradation because the receptacle darkens upon drying and obscures the faint diplodesmic veins. The normal veins sometimes anastomose irregularly in transitional part sterile, part fertile leaves (Walker, 1985a). I interpret this phenomenon as an early manifestation of the developmental pathways that convert the sterile veins into the reticulated diplodesmic veins.

Sporangia.—The sporangia of *Olfersia* resemble those described by Moran (1986) for *Polybotrya* with the exception of the occurrence in *Olfersia* of a single-celled gland near the base of the paraphysis. Plants from southeastern Brazil lack these glands, which occur in plants from other portions of the range (Fig. 4f, g). The function of these glands is unknown.

Spores.—The spores of *Olfersia* are monolete and generally 46–57 μm, relatively long compared with the spores of most other dryopteroid ferns (Tryon & Tryon, 1982). The exospore is smooth and overlain by a prominently folded perispore with a sparsely echinate surface. This sculpturing resembles that of *P. caudata*, although most species of *Polybotrya* have densely echinate perispores (Moran, 1986). Tryon and Tryon (1982, fig. 80.30) have a photograph taken with the scanning electron microscope of an *O. cervina* spore from Guatemala. Spores of plants from other Latin American countries show the same pattern of sculpturing (pers. obs.).

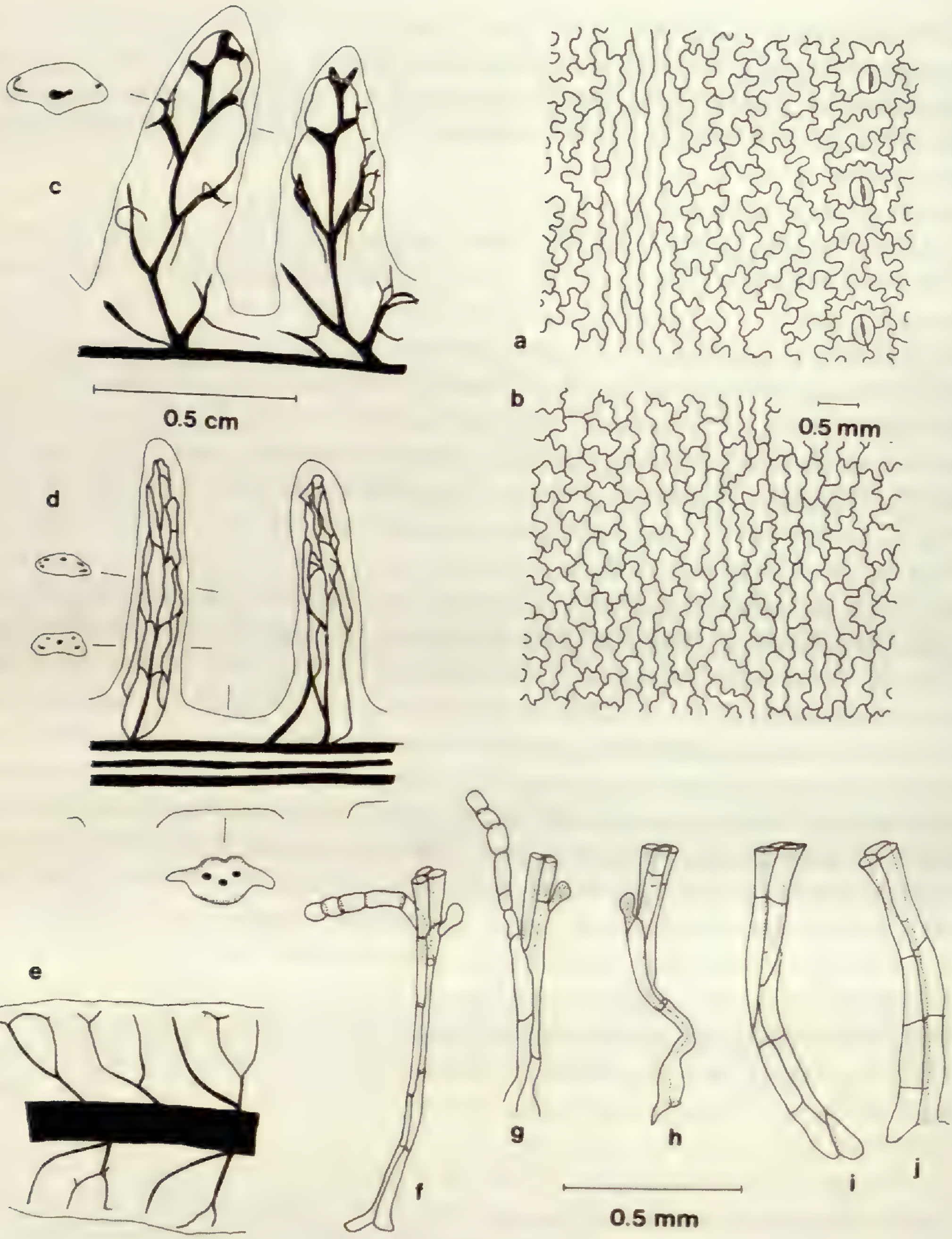


FIG. 4. Various anatomical characteristics of *Olfersia cervina*. a, abaxial epidermis; b, adaxial epidermis; c, d, e, diplodesmic veins of fertile pinnules, c and e show how the vein tips become modified to form the diplodesmic veins; g, h, sporangial stalks with glandular cell and paraphyses; i, j, sporangial stalks from southeastern Brazil lacking paraphyses and glandular cell. c: *Jürgens s.n.* (P). a, b, d: *Moran 2184* (F). e: *Glaziou 450* (P). f, g, h: *Smith 1284* (UC). i, j: *Mexia 4096* (UC).

CHROMOSOME NUMBERS

Three cytological studies report *Olfersia* as a sexual diploid. Walker (1966, fig. 40) first noted the chromosome number for *Olfersia cervina* as $n = 41$ from a plant collected in Jamaica. Smith and Mickel (1977) also found $n = 41$ for a plant collected on Trinidad and observed micronuclei present at meiotic teleophase

as in *Elaphoglossum*. Eight more plants of *Olfersia* from Trinidad were cytologically examined by Walker (1985b), and all showed $n = 41$. This base chromosome number, along with morphological and anatomical features, relates *Olfersia* to the dryopteroid ferns.

Relationship to *Polybotrya*

After studying the drastic changes in venation of what he called "anomalous" leaves, Walker (1985a) recently concluded that *Olfersia* is a simplified, derived member of *Polybotrya*. These leaves are cut to various degrees between the ample sterile leaves and the skeletonized fertile leaves. As the lamina becomes more reduced and divided, that is, more like the fertile leaves, the veins shorten and anastomose, forming a reticulum (see Walker's figs. 3–5). The pattern of this reticulum is similar to the venation of *Polybotrya* subgenus *Soromanes*, and Walker's suggestion that *O. cervina* is derived from *Polybotrya* is based upon this similarity.

Walker's "anomalous" leaves, however, actually represent leaves that are part sterile and part fertile. This transitional nature is the key to understanding why reticulate veins develop, as well as their taxonomic significance. The fertile leaves of *Olfersia* contain a highly specialized vascular supply to the sporangia—the diplodesmic veins, which form an elaborate network of delicate veins anastomosing irregularly beneath the sporangia (Fig. 4c, d, e). This special venation is derived from the veins of the sterile leaf, that is, the vein tips of the sterile leaf are converted into the diplodesmic veins (Fig. 4c, e). Thus, in a developmental sense, the veins of the sterile leaf and the diplodesmic veins of the fertile leaf are "homologous." This homology refutes the hypothesis that reticulate veins in part sterile, part fertile leaves of *Olfersia* indicate a relationship with *Polybotrya* subgenus *Soromanes*. Instead, reticulate veins in part sterile, part fertile leaves merely express a stage (albeit precocious) in the formation of reticulate, diplodesmic veins.

Pteridologists have often placed *Olfersia* in *Polybotrya* because both possess holodimorphic sterile and fertile leaves and creeping stems that are densely covered by scales. My research revealed additional similarities between the two genera (Table 1). But despite these similarities, important differences exist. Perhaps most significant is that *Olfersia* lacks the unique stem anatomy of *Polybotrya*. Moreover, *Olfersia* differs in two features of the lamina: the conform terminal pinna and venation unlike that of any dryopteroid fern. These differences justify keeping *Olfersia* distinct from *Polybotrya*.

Keeping these two genera distinct, however, does not resolve the question of their cladistic relationship. Two cladistic hypotheses seem probable (Fig. 5, Table 2). Hypothesis B, that *Olfersia* was derived from *Polybotrya*, requires that the unique stem anatomy of *Polybotrya*—the sclerenchyma sheath surrounding each meristele and the even, circular arrangement of meristeles—evolved and then was lost (Fig. 5, characters 9 & 10). It also requires a reversal in stem habit, from terrestrial and short creeping in the ancestor to long creeping in *Polybotrya*, back to short creeping and partially terrestrial in *Olfersia* (Fig. 5, character 8).

TABLE 1. Comparison of *Polybotrya* and *Olfersia*.

	SIMILARITIES
Stem indument:	Densely scaly
Vascular bundle arrangement in the petiole:	Mushroom-shaped
Sterile-Fertile leaves:	Holodimorphic
Paraphyses:	Present, unbranched, uniseriate
Sporangial stalks:	Two-rowed except three-rowed immediately below capsule
Indusium:	Absent
Diplodesmic veins:	Present
Chromosome number:	$n = 41$

	DIFFERENCES	
	Polybotrya	*Olfersia*
Leaf apex:	Pinnatifid	Conform, similar to lateral pinnae
Venation:	Various, but not as in *Olfersia*	Numerous, fine, long and parallel
Submarginal connecting strand:	Absent (present in *P. polybotryoides*)	Present
Laminar scales:	Proscales and polyseriate scales	Proscales only
Hairs on the axes:	Generally present	Absent
Fertile and sterile leaf length:	Fertile leaf shorter than the sterile, but longer in the two terrestrial species	Fertile leaf longer than sterile leaf due to lengthened petiole
Fertile leaf production:	Only on scandent portion of stem	On scandent or terrestrial stems
Seasonality of fertile leaves:	Seasonal and continual	Continual throughout the year
Perispore sculpturing:	Usually spiny, rarely smooth	Smooth
Meristele arrangement:	Circular	Irregular
Sclerenchyma sheath surrounding meristele:	Present	Absent
Stem habit:	High-climbing (two spp. terrestrial)	Short-creeping, terrestrial or climbing for short distances

In short, hypothesis B requires three reversals. Hypothesis A, that the two genera evolved from a common ancestor that lacked the unique stem anatomy but had dimorphic leaves, requires no reversals. Therefore, hypothesis A is favored. Moran (1986) presented evidence that *Cyclodium* is the most likely ancestor of both genera.

TAXONOMIC TREATMENT

Olfersia Raddi, Opusc. Sci. Bologna 3:283 tab. 11., fig. b. 1819.—*Acrostichum* subg. *Olfersia* (Raddi) Hooker, Sp. Fil. 5:254. 1864.—TYPE: *O. corcovadensis* Raddi.

Dorcapteris Presl, Epim. Bot. 166. 1849.—TYPE: *Osmunda cervina* L.

A monotypic genus, with the characteristics of its single species.

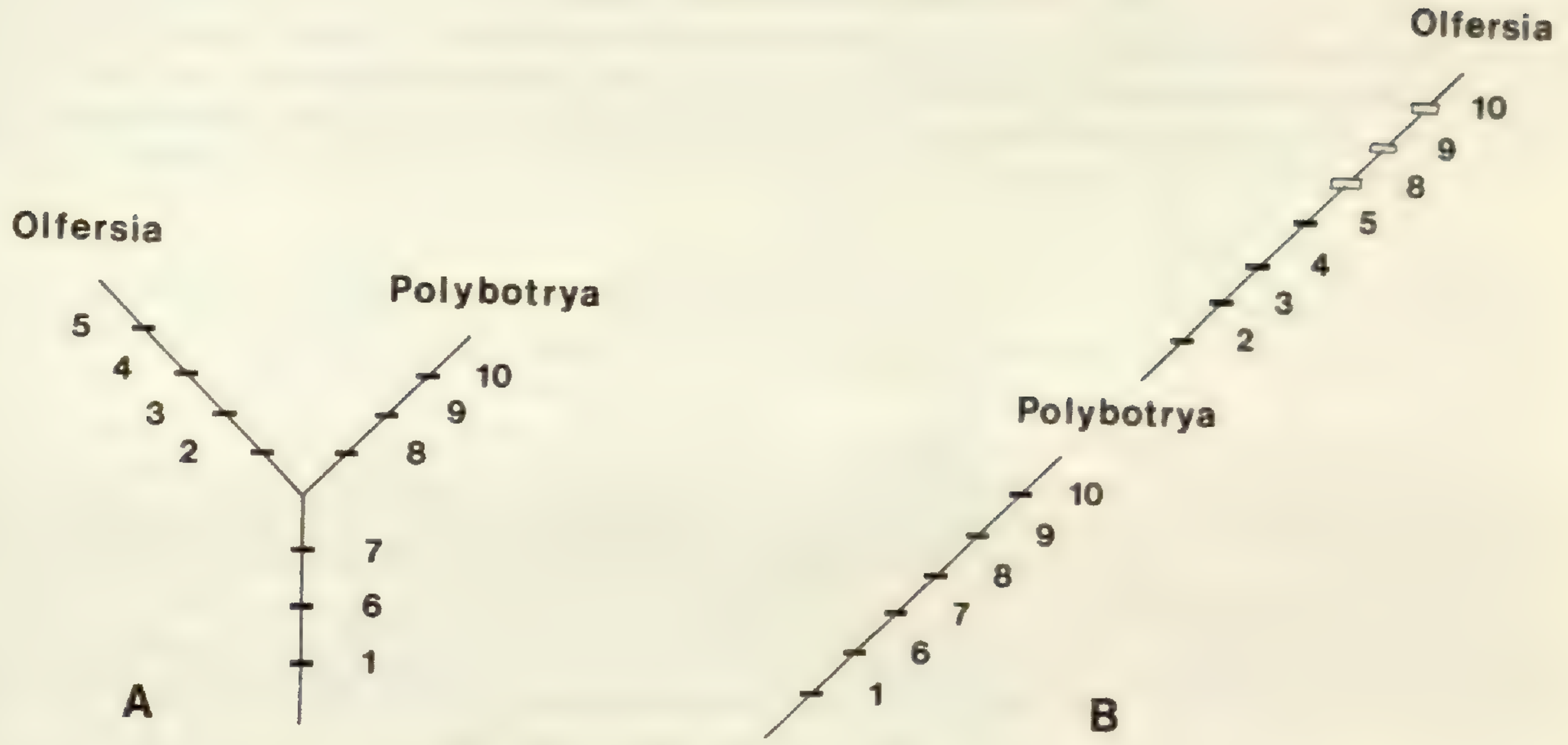


FIG. 5. Alternative hypotheses concerning the evolutionary relationship of *Olfersia* and *Polybotrya*. The numbers represent derived character states shown in Table 2. The open rectangles in hypothesis B represent character state reversals.

Olfersia cervina (L.) Kunze, Flora 7:312. 1824. *Osmunda cervina* L., Sp. Pl. 2: 1065. 1753.—*Acrostichum cervinum* (L.) Swartz, Syn. Fil. 14. 1806.—*Polybotrya cervina* (L.) Kaulfuss, Enum. Fil. 55. 1824.—*Dorcapteris cervina* (L.) Presl, Epim. Bot. 167. 1851.—LECTOTYPE: (chosen by Proctor, Fl. L. Antill. 2: 223. 1977): Plumier, Tract. Fil. Amer., pl. 154 (1705), illustrating a plant from Martinique. Plate 154 is mounted on herbarium sheets at F and GH.

Olfersia corcovadensis Raddi, Opusc. Sci. Bol. 3:283, tab. 11, fig. b. 1819.—*Polybotrya corcovadensis* (Raddi) Spreng., Syst. Veg. 4:33. 1827.—*Polybotrya raddiana* Link, Hort. Berol. 2:134. 1833 (nom. superf. for *P. corcovadensis*).—TYPE: Brazil, Rio de Janeiro, Mt. Corcovado, *Raddi* (not seen, but Raddi's tab. 11 certainly represents this species).

Polybotrya cervina (L.) Kaulfuss forma *transitoria* Rosenstock, Hedwigia 46:156. 1907.—TYPE: Brazil, Santa Cruz, Piccado Nova, *Jürgens & Stein 69* (NY!, UC!).

Stem short-creeping, primarily terrestrial, occasionally short climbing, in cross-section with numerous scattered dark sclereids, the meristeles irregularly shaped and arranged, not surrounded by a dark sclerenchyma sheath; *scales* linear, to 25 × 2 mm, yellowish to golden, often turning brown upon drying, the margins denticulate to nearly entire, the base truncate, attached along its length. *Sterile leaves* subdistant, usually 0.5–1.2 × 0.3–0.5 m; *petiole* shorter than the lamina, 20–50 cm long, scaly at base, with scales similar to those on the stem; *lamina* pinnate with a conform terminal pinna, thick, subcoriaceous, apparently glabrous, but actually with minute, <0.1 mm long, appressed proscales; *pinnae* ovate-lanceolate to lanceolate, commonly 15–30 × 3–8 cm, 4–12 pairs, short-stalked, continuous (not articulate) to the rhachis, the margins entire, the base unequal, with the basiscopic side excavate and the acroscopic side broadly rounded, the apex acute to acuminate; *veins* forking near or at the base, parallel, ca. 1 mm

TABLE 2. Characters and Character States Used in the Cladistic Analysis (Fig. 5) of *Olfersia* and *Polybotrya*. Character State Polarities were Determined by Comparison to Closely Related Outgroup Genera, such as *Arachniodes*, *Cyclodium*, *Dryopteris*, *Maxonia*, and *Polystichopsis*.

Character	Ancestral condition	Derived condition
1. Sterile & fertile leaves:	Monomorphic	Dimorphic
2. Leaf dissection:	Decompound (3-pinnate)	1-pinnate
3. Apex shape:	Pinnatifid	Conform
4. Vein groups:	Pinnate groups	Not pinnate
5. Veins:	Free	Connected at tips
6. Sporangial stalks:	Glabrous	Paraphysate
7. Diplodesmic veins:	Absent	Present
8. Stem habit:	Terrestrial and short-creeping	Climbing and long-creeping
9. Sclerenchyma sheath surrounding meristeles:	Absent	Present
10. Meristele arrangement:	Irregular	Circular

apart, slightly oblique to the costae, connected by a submarginal connecting vein (at times difficult to see in dried material); *axes* nearly glabrous, but with slender, inconspicuous, appressed, multicellular hairs (actually reduced scales), the cells often flattened with reddish cross walls, these hairs intermixing and intergrading with appressed, narrow, scattered scales; *grooves* shallow, glabrous, not decurrent. *Fertile leaves* 1- or 2-pinnate, produced freely on terrestrial as well as scandent stems, more erect than the sterile leaves and taller due to the lengthened petiole; *petiole* longer than the petioles of the sterile leaves, usually 40–85 cm long; *pinnae* pectinate, linear, 7–20 cm long; *pinnulus* 0.5–1 × 0.1–0.3 mm, set at right angles to the costa, broadly adnate and joined by a narrow wing of tissue; *sori* exindusiate, spreading along the axes; *sporangial stalks* paraphysate and with a single glandular cell, the paraphysis hairlike, 3–6 celled, the apical cell clavate-glandular, but the stalks in plants from southeastern Brazil are eglandular and non-paraphysate; *spores* monolete, echinulate, (40)46–57(60) μm. $n = 41$.

Selected Specimens Examined.—MEXICO. **Oaxaca:** Distrito Ixtlán, 29 km S of Valle Nacional, 80 km N of Ixtlán de Juárez, trail E of Rte 175 at Campamento Vista Hermosa toward Ladú. *Mickel 6384* (NY), *6399* (NY). **Veracruz:** Mpio. Hidalgotitlán, afluente del Río Las Cuevas, *Wendt et al. 3846-A* (NY). BELIZE. Middlesex, *Schipp 402* (F, GH, MICH, NY, UC, US, Z); Stann Creek, Mt. Cow Valley, *Gentle 3528* (MICH). GUATEMALA. **Alta Verapaz:** Cubilquitz, *von Türckheim 418* (P, US) *4031* (GH, LIL), *8044* (GH). **Izabal:** Near Puerto Barios, *Standley 72823* (F). **Petén:** Dolores, km 85 W of Machaquila Road, *Contreras 2648* (US). **Quiché:** Finca Chaila, "Zona Reyna," *Skutch 1805* (P); no locality, *Skutch 1805* (GH). **Prov. Unknown:** no locality, *Godman & Salvin 99* (BM). HONDURAS. **Atlántida:** near Ceiba, *Dyer 208* (NY, US). **Cortés:** along Lake Yojoa, ca. 6 km N of Rancho Agua Azul, *Williams & Molina 17787* (US). **Gracias a Dios:** Camp Iro, 2 mi NW of Bulebar, *Saunders 1117* (NY). **Santa Barbara:** Cerros de la Leona, inmediaciones El Mocho, *Gómez 6895* (CR). NICARAGUA. **Zelaya:** near Río Okanwas, 12 km E of Rosita, *Neill 4481* (CR). COSTA RICA. **Alajuela:** La Palma de San Ramón, *Brenes 5768* (F). **Cartago:** Peralta, *Lankester 7925* (NY); 2.6 km W of Paraiso, *Armond 155* (MO). **Cocos Island:** Chatham Bay, *Jiménez 3157* (CR, F, NY, US). **Limón:** 7 km S of Bibri, *Gómez et al. 20343* (MO). **Puntarenas:** Sabanillas de Limoncito, Coto Brus, *Gómez 22034* (CR, MO). **San José:** 2.7 km SE of bridge over Río Pacuare, 10 km SE of San Isidro, *Wagner & Gómez 79637* (CR). **Prov. Unknown:** Navarito, *Lankester 728* (F, US). PANAMA. **Chiriquí:** vicinity of Gualaca, ca. 8.5 miles from Planes de Hornito, La Fortuna on road to damsite, *Antonio 5068* (MO). **Coclé:** Los Ped-

regales, ridge between Río Blanco del Norte and Río Caño Sucio, *Knapp & Dressler 3786* (MO). **Darién:** Serranía de Pirre, 9–10 km due N of Alto de Nique, *Croat 37859* (MO). **Veraguas:** valley of Río Dos Bocas on road between Alto Piedra (above Santa Fe) and Calovebora, *Croat 27421* (MO).

CUBA. **Oriente:** Montes United Fruit, Nícaro, Mayarí, *Acuña & Zayas 19620* (US). **Las Villas:** banks of Yayabo River, Bauao Hills, *Leon & Clement 3975* (NY). **Santiago:** Santiago, *Linden 2188* (P). JAMAICA. **Portland:** John Crow Mts., *Crosby et al. 975* (MICH). **St. Catharine:** Mt. Diabolo, *Hespenheide 1396* (GH). **St. Thomas:** mountain trail between House Hill and Cuna Cuna Gap, *Maxon 8918* (NY), *8976* (NY). **Westmoreland:** Cho-Cho Gully, 4.5 miles ESE of Bluefields, *Wilson & Webster 507* (GH). PUERTO RICO. Vicinity of Hollymount, Mt. Diabolo, *Maxon 2220* (NY); El Yunque, *Scamman 6540* (GH); Luquillo National Forest, La Mina Falls trail, *Howard et al. 15570* (GH). HAITI. Massif du Nord, Morne Rhesnau, *Ekman 4394* (US). DOMINICAN REPUBLIC. **Barahona:** Montiada Nueva, SE of Polo, *Howard & Howard 8538* (BM, GH, NY, US). **San Francisco de Macoris:** Lo Bracito, *Abbott 2197* (US). **Santiago:** Loma Bajita, District San José. *Valeur 1006* (NY, US). **Santo Domingo:** Cordillera Central, La Cumbre, Arroyo Los Guananitos, *Ekman 12364* (US).

MARTINIQUE. Pilon du Vauchoy, *Plee s.n.* (P); no locality, *Belanger 25* (F, P); Calebasse, 1868, *Hahn 2* (BM); forests of Camp Colson, *Duss 1502* (NY, P, US). DOMINICA. Lisdara, *Hodge 100* (NY, US); Mt. Diablotiri, *Lloyd 894* (NY); Waterfall, Roseau Valley, *Lloyd 802* (NY, US). GUADELOUPE. Cascade Vauchelet, *Rodriguez 6738* (P); *Duss 872* (P); forests of Bains-Iaunes, *Duss 4140* (NY, P); no locality, *L'Herminier s.n.* (NY, P). ST. LUCIA. Great Piton, *Ramage s.n.* (BM); Roseau-Milette Ridge, *Box 514* (BM, US). ST. VINCENT. Mountain tops, *Smith & Smith s.n.* (BM); *Eggers 6766* (F); Mts. above Chateaubelair River, *Morton 5250* (US); along Chateaubelair River, *Morton 5412* (US); Cumberland Mts., *Morton 5909* (US). GRENADA. No locality, *Sherring 177* (NY, US). ST. CHRISTOPHER. Mt. ridge N of the Crater, *Box 288* (BM, US); Nine Turn Gut, *Box 361* (BM, US); 1889, *Berkeley s.n.* (NY). MONTSERRAT. *Shafer 377* (F, NY, US); *Shafer 763* (NY, US). TRINIDAD. 2.75 miles up Aripo Valley, S side of N Range, *Jermy 2625* (BM); 3 miles up Caura Road, by river, *Jermy 2101* (BM); Maracas Falls trail, *Fay 224* (BM).

FRENCH GUIANA. Arataye River, tributary of Approuague, *Oldeman 2991* (CAY, NY); région de Paul Isnard, Massif du Décou Décou, *Cremers 7927* (CAY, Z); Massif des Emerillons, *De Granville 3901* (CAY); Sommet Tabulaire, ca. 45 km SE de Saul, *Cremers 6393* (CAY, Z); entre Saut Mais et Pic Matécho, 30 km ENE de Saul, *de Granville 3095* (CAY); région de Saul, *de Granville 3286* (CAY). SURINAM. Nassau Mts., *Maguire & Maguire 40703* (NY); Nassau Mts., Marwijne River, *Maguire et al. 39085* (NY); Lely Mts., SW plateaus, *Lindeman et al. 465* (NY, Z); region of Mt. Raywa, *Jenman s.n.* (NY); Dist. Brokopondo, Nat. Res. Brownsberg, *Tjon-Lim Sang & Wiel 51* (US). GUYANA. Cuyuni River, Arawak Matope, *Tutin 380* (BM). VENEZUELA. **Aragua:** prope coloniam Tovar, *Fendler 426* (GH, MO). **Barinas:** Dtto. Bolívar, 1 km E of La Soledad, *Smith 1374* (UC). **Bolivar:** Sierre de Lema, Cabeceras de Río Chicanán, 80 km (en línea recta) al SW de El Dorado, *Steyermark 89382* (VEN). **Falcón:** Sierra de San Luis, Montaña de Paraguariba, approxamadamente 5 km E del Hotel Parador, *Steyermark 99319* (NY, VEN). **Lara:** Dtto. Palavencino, road to Parque Nacional Terepaima, ca. 10–11 km SE of town of Río Claro, *Smith 1284* (UC). **Mérida:** Dtto. Sucre, Estanquez, Paramo Las Coloradas, *Ortega & Marcano-Berti 2112* (PORT). **Miranda:** Sta. Teresa-Altagracia de Orituco, *Aristeguieta 1761* (VEN). **Neuva Esparta:** Isla Margarita, *Johnston 152* (F, GH, NY, UC). **Sucre:** Dtto. Marino & Arisemendi, Península de Paria, *Steyermark et al. 121554* (MO). **Tachira:** vicinity of Las Minas, 16 km SE of Santa Ana, *Steyermark & Liesner 118892* (MO, VEN). **Trujillo:** Dtto. Boconó, 33.5 km SE of Boconó, road to Guaramacál, *Smith 1542* (MO, PORT, UC). **Yaracuy:** Dtto. Bruzual, Montaña de María Lionza, *Steyermark et al. 125015* (UC). **Zulia:** ca. 55 km SW of Machiques by air, Aricuaisa, *Liesner & Gonzáles 13172* (MO). **Prov. Unknown:** Regnesiva del Diablo, cabeceras del Río Castaño, *Pittier 14042* (F, VEN). COLOMBIA. **Boyaca:** valle del Río Cusiana, entre Pajarito y Aguazul, Vereda San Benito, *Murillo 1412* (COL), *1413* (COL), *1420* (COL), *1423* (COL). **Chocó:** NW side of Alto del Buey, *Lellinger & de la Sota 287* (COL, CR, US). **Caqueta:** Apaporis River, Cerro Castillo, *Schultes 5660* (GH). **Guajira:** Serranía de Macuira, Cerro Huararech, *Sugden 30* (UC). **Magdalena:** Santa Marta, *Smith 1075* (COL, F, MICH, MO, NY, PH, UC, VT). **Nariño:** Municipio de Ricaurte, entre La Planada y Pialapi, *Mora 4021* (COL). ECUADOR: **Carchi:** Peñas Blancas, 20 km below Maldonado on the Río San Juan, *Madison et al. 4616* (F). **Morona-Santiago:** Shuinia Nait, small mountain ridge ca. 8 km SE of Misión Bomboiza, *Holm-Nielsen et al. 4387* (AAU). **Napo:** road Baeza–Lago Agrio, S of Finca La Cascada, ca. 92 km from Lago Agrio, *Øllgaard et al. 35677* (AAU,

Q). PERU. **Amazonas:** Prov. Bagua, 43 km (by road) NE of Chiriaco, *Barbour 4514* (MO). **Cuzco:** Prov. La Convención, *Dudley 10324* (GH). **Huánuco:** ridge E of Tingo María, *Allard 22301* (GH, US), *22304* (US), *22523* (US); Prov. Leonicio Prado, Dtto. Rupa Rupa, cerca Cerro Quemado, E of Tingo María, *Schunke 10491* (MO). **Madre de Dios:** Pantiacolla, serranía across Río Alto Madre de Dios from Shintuya, *Gentry et al. 27366* (MO). **Puno:** Prov. Carasaya, *Vargas 18936* (GH). BOLIVIA. **La Paz:** Río Chimate, *Tate 513* (NY); Ipurima, *Williams 1201* (GH, NY, US); Hacienda Simaco sobre el camino a Tipuani, *Buchtien 5233* (F, MO, NY, US, Z). **Cochabamba:** Prov. de Chapare, road to San Onofre, 3.3 km N of road to Villa Tunari at point 97.5 km from Cochabamba, *Foster 79-140* (UC). BRAZIL. **Espíritu Santo:** "Voyage d'Auguste de Saint-Hilaire," no collector #342 (P); Santa Barbara de Caparão, near waterfall above village, *Mexia 4096* (US, UC). **Mato Grosso:** Gorge of Veu da Noiva, Chapado do Guimarães, *Prance et al. 19114* (NY). **Pará:** Serra dos Carajás, "Azul," near camp at Serra Norte, *Daly et al. 1803* (GH, NY). **Paraná:** Arbatão, *Dusen 13642* (NY). **Rio de Janiero:** Tijuca, *Alston 9009* (BM). **Rio Grande do Sul:** Sta. Cruz. *Jürgens 69* (NY, UC). Santa Catarina: Santo Antonio, *Rohr 1035* (LIL). São Paulo: Iguape, Morro dos Pedras, *Brade 2708* (PH), *7708* (NY, UC). Prov. Unknown: no locality, *Martius 375* (MO).

EXCLUDED TAXA

See Christensen (1906) for a complete list of excluded taxa, most of which are species of *Elaphoglossum* and *Stenochlaena* placed in the genus by Presl (1851).

ACKNOWLEDGMENTS

I thank Dr. Kenneth R. Robertson (Illinois Natural History Survey, Champaign) for his help and encouragement with all aspects of this study. Most of the work for this monograph was done in the herbarium at the Illinois Natural History Survey, Champaign, and I am greatly indebted to the Survey for its support and use of its facilities. Mr. Bill N. McKnight, also of the Survey, assisted with the reproduction of the illustrations.

This work was done while I was a doctoral student in the Department of Plant Biology, University of Illinois, Urbana. I thank the Department for its financial support.

My first field trip, to Costa Rica, was made on funds awarded by the Tinker Foundation. A Doctoral Dissertation Improvement Grant (#83-06990) from the National Science Foundation provided financial support for three additional collecting trips in Latin America and for a semester of study at Harvard University.

Correspondence and discussion with the following pteridologists gave insight into various aspects of the biology of *Olfersia*: Dr. Alan R. Smith (University of California, Berkeley), Mr. Robert G. Stolze (Field Museum of Natural History, Chicago, IL), Dr. Rolla M. Tryon and Dr. Alice F. Tryon (Harvard University, Cambridge, MA), and Dr. Warren H. Wagner, Jr. (University of Michigan, Ann Arbor).

I am grateful to the directors and curators of the following herbaria for the large quantities of valuable material they made available to me, often for a considerable period of time: A, AAU, AMAZ, B, BM, CAY, COL, CR, F, G, GH, L, LIL, LPB, MICH, MO, NY, P, PH, PORT, Q, QCA, RB, UC, US, USM, VEN, VT, Z. Unfortunately, *Olfersia* at K could not be examined because their policy precludes loaning specimens for use by graduate students (G. L. Lucas, *in litt.*, 1983).

LITERATURE CITED

BRADE, A. C. 1971. O gênero *Polybotrya* no Brazil. Bradea 1:57–67.

CHRISTENSEN, C. 1905–1906. *Index Filicum*. Copenhagen: H. Hagerup.

———. 1913. A monograph of the genus *Dryopteris*, part 1. Kongel. Danske Vidensk. Selsk. Skrift, Naturvidensk. Afd., VII. 10:55–282.

HOLTTUM, R. E. 1978. *Lomariopsis* group. Flora Malesiana, II. 1:255–330.

———. 1984. Studies of fern genera allied to *Tectaria*, I. A commentary on recent schemes of classification. Fern Gaz. 12:313–319.

HOOKER, W. J. 1864. *Species filicum*, vol. 5. London: William Pamplin.

KAULFUSS, G. F. 1824. *Enumeratio filicum*. Leipzig: Cnobloch.

LINK, H. F. 1833. *Hortus regius botanicus Berolinensis, 2*. Berlin: Reimer.

LINNAEUS, C. 1753. *Species plantarum*. Stockholm: Salvi.

MEHRA, P. N. and T. C. MITTAL. 1961. Significance of internal glands in relation to filicin. Pl. Med. 9:189–199.

MORAN, R. C. 1986. Monograph of the neotropical fern genus *Polybotrya*. Ph.D. dissertation, Univ. of Illinois at Urbana-Champaign.

PETIVER, J. 1712. *Pterigraphia americana*. London.

PICHI SERMOLLI, R. E. G. 1977. Tentamen pteridophytorum genera in taxonomicum ordinem redigendi. Webbia 31:313–512.

PLUMIER, C. 1705. *Traité des fougères de l'Amerique*. Paris: Imprimerie royale.

PRESL, K. B. 1851. *Epimeliae botanicae*. Prague: Haase.

PROCTOR, G. R. 1977. Pteridophyta. *Flora of the Lesser Antilles*. vol. 2, ed. R. A. Howard. Jamaica Plain, Massachusetts: Arnold Arboretum.

RADDI, J. 1819. *Synopsis filicum Brasiliensium*. Opusc. Sci. Bol.

SMITH, A. R. and J. T. MICKEL. 1977. Chromosome counts for Mexican ferns. Brittonia 29:391–398.

SMITH, J. 1875. *Historia filicum*. London: MacMillan.

SPRENGEL, C. 1827. *Caroli Linnaei, systema vegetabilium*, ed. 16. Gottingen: Dieterich.

SWARTZ, O. 1806. *Synopsis filicum*. Kiel: Bipliop. Nov. Acad.

TRYON, R. M. 1970. Development and evolution of fern flowers of oceanic islands. Biotropica 2: 76–84.

——— and A. F. TRYON. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.

WAGNER, W. H., Jr. and D. M. JOHNSON. 1983. Trophopod, a commonly overlooked storage structure of potential systematic value in ferns. Taxon 32:51–63.

——— and F. S. WAGNER. 1977. Fertile-sterile leaf dimorphy in ferns. Gard. Bull. Straits Settlem. 30:251–267.

WALKER, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Trans. Roy. Soc. Edinburgh 66:169–237.

———. 1972. The anatomy of *Maxonia apiifolia*: a climbing fern. Fern Gaz. 10:241–250.

———. 1985a. Anomalous fronds and venation in *Polybotrya cervina*. Fern Gaz. 13:13–16.

———. 1985b. Cytotaxonomic studies of the ferns of Trinidad 2. The cytology and taxonomic implications. Bull. Brit. Mus. (Nat. Hist.) Bot. 13:149–249.

WILLDENOW, C. L. 1810. *Caroli a Linné species plantarum*, ed. 4, vol. 5. Berlin: Nauk.

APPENDIX: LIST OF EXSICCATA

The following is a complete list of all numbered collections seen; unnumbered collections are not listed.

Abbott 1951, 1980, 2197. Acuña & Zayas 19620. Alain 3236. Allard 22301, 22304, 22523. Alston 5825, 9009. Anderson & Sternberg 3321. Antonio 3715, 5068. Aristeguieta 1761. Armond 155.

Barbour 2625, 4514. Barnard et al. 428, 505. Belanger 25. Blomquist 11928. Box 288, 361, 514. Brade 373, 2708, 7708. Brenes 5768, 16236, 16411, 33122. Britton 780, 2249. Britton & Bruner 7592. Britton & Cowell 2210. Britton & Marble 1081. Britton & Shafer 2066. Britton & Wilson 5280. Britton et al. 1242, 5246, 2599. Buchtien 1052, 2240, 5233. Burch 1357. Burger & Antonio 11115, 11272. Burger & Burger 8016. Burger & Matta 4170, 4554. Burger & Stolze 5838.

Chacón 11. Chacón & Herrera 1683. Chrysler & Roever 5240. Clement 982, 6666. Clute 296. Contreras 2648. Cook & Doyle 12. Cook & Griggs 567. Cremers 6393, 7927. Croat 764, 23219, 25627, 26530, 27421, 33285, 35812, 36794, 37859. Crosby et al. 975.

Dale 23. Daly et al. 1803. Daniels & Jonker 1044. Donnell-Smith 6938. Dudley 10324. Dusén 78, 8185, 13642. Duss 872, 1502, 4140. Dyer 208.

Eggers 1040, 5207, 6766. Ekman 4356, 4394, 6759, 12364. Ernst 2055. Evans & Bowers 3303.

Fay 187, 393. Fendler 59, 426. Fielder 156. Fisher 42. Folsom 5916. Folsom & Edwards 3377. Foster 97-140. Foster & Foster 1290. Fournier 309. Fuertes 944.

Galeotti 253. Gastony et al. 387, 648. Gaudichaud 48. Gentle 3528. Gentry & Glewell 6958, 6959. Gentry et al. 27366. Glaziou 450. Godman & Salvin 99. Gómez 3351, 6895, 7123, 19439, 22034, 20343. Granville, de 3095, 3286, 3901.

Hahn 2. Hart 202, 228, 6307. Hespenheide 1396. Hess 240, 7040. Hioram 249, 2422. Hodge 100, 101. Hodge & Hodge 1715, 1785, 1976, 2874, 3563. Holdridge 5148. Holm-Nielsen et al. 4387. Hombersley 105. Howard 11152, 12215. Howard & Howard 8538. Howard et al. 15570.

Jermy 2101, 2625, 2848, 10857, 10988. Jiménez 1377, 2106, 3157. Johnston 152. Jürgens 69.

Kennedy 1961. Klawe 1546. Knapp 5342. Knapp & Dressler 3786. Koptur 335.

Lankester 728, 7925. Laurito 8802. Lellinger 396, 550. Lellinger & de la Sota 287. Lent 1424. Lent et al. 3363. Leon 3230. Leon & Clement 3975, 6611. Leon & Roca 7991. Leon & Seifriz 78212. Leon et al. 10421. L'Herminier 19. Liesner & González 13172. Lindeman et al. 465. Linden 2188. Liogier 10080. Lloyd 802, 894. Lutz 1450. Lutzelburg 7080.

Madison et al. 4616. Maguire & Maguire 40703. Maguire et al. 39085. Martius 375. Maxon 2220, 2280, 2511, 4179, 8918, 8976, 9037, 9045. Maxon & Killip 452, 754. McCallum 105. Mexia 4096. Mickel 3425, 3468, 3043, 6384, 6399. Mora 4021. Moran 2181, 2184. Morely & Whiteford 637. Morton 5250, 5412, 5909. Murillo 1412, 1413, 1420, 1423.

Nee 9793. Neill 4481.

Ocampo 1082, 1996. Ohaus 98. Oldeman 2991. Øllgaard et al. 35677. Ortega & Aymard 1562. Ortega & Gonzalez 355. Ortega & Marcano-Berti 2112.

Pablo 36. Pabst 4771. Pipoly 5302, 5916. Pittier 1824, 12369, 14042, 16400. Pollard et al. 236. Prance et al. 19114. Proctor 3968, 18946, 21730.

Reitz & Klein 3081. Rodriguez 6738. Rohr 235, 1035. Rose & Russel 21261.

Saunders 1117. Scamman 5982, 5983, 6540, 6544, 7145, 7481, 7719, 7997, 8149. Schipp 402, 932. Schmalz 183. Schultes 5660. Seifriz 322. Shafer 377, 763, 3229, 3506, 3570, 4441, 8620, 8819. Sherring 177. Sitensis 1377. Skutch 1805. Smith, A. R. 1284, 1374, 1542. Smith, H. H. 1075. Smith, J. D. 6938. Smith, L. B. & Brade 2207. Snodgrass & Heller 959. Solomon et al. 6581. Sota, de la 758. Spannagel 326. Standley 1939, 7957, 72823. Standley & Valerio 47000, 48521. Steeves & Ray 461, 470. Stevens & Krukoff 6706, 7101, 12214. Steyermark 75597, 89159, 89382, 99319. Steyermark & Agostini 52931. Steyermark & Liesner 118892. Steyermark & Rabe 71722, 71723, 96371. Steyermark et al. 95827, 121554, 125015. Stimson 3891. Sugden 30. Svensen 342.

Tate 513. Tjon-Lim Sang & Wiel 51. Tschudi 163. Turckheim, von 418, 4031, 8044. Tutin 380.

Underwood 1249, 1257, 2716, 2888.

Valerio 1084, 33123. Valeur 360, 770, 1006. Vareschi 5771. Vargas 18936.

Wagner & Gómez 97637. Watt 96. Weber 6129. Weddel 650. Wendt et al. 3846. Werff, van der & Wingfield 3421. Williams 1201. Williams & Molina 17787. Wilson 106. Wilson & Webster 448, 507. Wright 784.

Yunker 18544.

American Fern Journal 76(4):179–183 (1986)

Trichomanes in Florida

CLIFTON E. NAUMAN
Fairchild Tropical Garden, 10901 Old Cutler Road, Miami, FL 33156

The genus *Trichomanes* L. is represented in Florida by five species in two subgenera. Their increasing rarity and a lack of ecological information on these species warrants bringing renewed attention to the genus in Florida. Toward that end, I have provided diagnostic and distributional information as well as a key for the Florida taxa. This article is intended to update the previous literature and to subject the information to public critique prior to its inclusion in the pteridophyte treatment for the flora of Florida. For synonymy the reader should consult Wessels Boer (1962) and Proctor (1977, 1985); for the subgeneric treatment of *Trichomanes* see Morton (1968). The key and distributions were compiled from field populations and representative specimens at FLAS, FTG, NY, and USF.

KEY TO THE FLORIDA SPECIES

1. False veins (those not connected to other veins) absent; lamina without marginal, castaneous, acicular trichomes in stellate clusters, mostly pinnatifid with broad segments, never simple, entire leaf usually more than 5 cm long (subg. *Achomanes* Presl) ..... *T. holopterum*
1. False veins present; lamina with marginal castaneous, acicular trichomes in stellate clusters, simple or pinnatifid with narrow segments, entire leaf usually less than 5 cm long (subg. *Didymoglossum*) 2
   2. Veins pinnate; lamina pinnatifid ..... *T. krausii*
   2. Veins flabellate; lamina simple or lobed 3
3. Midrib extending to apex of the lamina; involucres fully immersed; lips immersed, indistinct from lamina tissue, but flaring and undulate, usually without a dark line of marginal cells ..... *T. petersii*
3. Midrib not extending past the middle of the lamina, or absent; involucres fully immersed or not; lips not immersed, distinct from lamina tissue, usually with a dark line of marginal cells 4
   4. Veins of uniform width to their apices .... *T. punctatum* ssp. *floridanum*
   4. Veins widening at their apices ..... *T. lineolatum*

Trichomanes holopterum Kunze—In addition to the key characters, this species can be distinguished from the other taxa by its erect to ascending rhizomes, generally exserted receptacles, and fully immersed involucres lacking distinct lips.

Distribution and habitat.—Monroe and Collier Counties, Florida, and the Antilles. Epiphytic plants on trees, logs, and stumps; generally in moist, relatively protected habitats; Florida populations are known only from cypress swamps. Sporulates all year.

This species was first collected in Florida in 1964 by R. and R. Stone in the Big Cypress Swamp, Collier County (Delchamps, 1966). Lakela and Long (1976) cited only Monroe County for the species. The occurrence of the species in two large counties is perhaps misleading since the stations for it in Collier and Monroe are relatively close to their common border. Visits to the populations in early 1986 indicated that plants are still present in relatively large numbers. Available habitat was abundant nearby and it is reasonable to expect more stations to be found with continued field work.

The present populations compare well with the description given by Farrar and Wagner (1968), indicating a fairly stable existence (at least locally). Farrar and Wagner described the gametophytes as more common and covering a larger area than the sporophytes; this is true for the present populations. It is possible that the gametophytes are more protected from periodic dry conditions by being more appressed to the substrate than are the emergent sporophytes. This suggestion requires more field study, but may explain the greater abundance of the gametophytes at these localities. The gametophytes are frequently embedded in moss and liverwort colonies; any effects on the populations due to competiton with the latter are unknown.

Trichomanes krausii Hooker & Greville—*Trichomanes krausii* resembles *T. holopterum* in having pinnatifid leaves, but can be distinguished by its narrower segments, castaneous, acicular, marginal (often reflexed) trichomes, and a distinct involucre lip that is not completely immersed in the leaf tissue.

Distribution and habitat.—Dade County, Florida, and the Antilles south to continental tropical America; epiphytic or rarely epipetric plants in pockets of humus on limestone, in moist forested habitats. Sporulates all year.

Trichomanes krausii was first collected in Florida in 1903 by J. K. Small and J. J. Carter in Dade County. Plants were subsequently collected by Small from Nixon-Lewis Hammock, Camp Longview, and Silver Palm, and collected by A. A. Eaton south of Cutler. The plants are absent now from most of these localities, probably as a result of habitat disturbances. The Nixon-Lewis Hammock for example, has been bulldozed (P. Adams, pers. comm.).

Plants are currently known to occur in Castello and Fuchs hammocks, at most only a few miles from the Silver Palm location reported by Small (1938). The population at Castello Hammock is represented by a single colony of about three square decimeters. A search was not made for gametophytes, but they may occur apart from the sporophytes at these stations and possibly in at least one adjacent hammock (Ross Hammock) in which the sporophytes are apparently absent. If the gametophytes are persistent and as numerous as those of *T. holopterum*, it is possible that new locations for the plants may be found.

Trichomanes lineolatum (Bosch) Hooker in Hooker & Baker.

Distribution and habitat.—Dade County, Florida (now apparently extirpated), the Antilles, Venezuela, and Brazil; epipetric or rarely epiphytic plants in moist forested habitats. Wessels Boer (1962) reported the species only on limestone. Sporulates all year.

Lellinger (1985) treated *T. lineolatum* as extirpated in Florida. This appears to be the case, but I have included the species here in order to facilitate its rediscovery by other workers. The species is easily confused with *T. punctatum* ssp. *floridanum*, which is extant in Castello Hammock and adjacent Ross Hammock. It is possible that *T. lineolatum* has been overlooked in this locality because of its similarity to *T. punctatum* ssp. *floridanum*. (The key characters easily separate the two taxa, but require a 10 power handlens.)

This species was first collected in Florida in 1906 by J. K. Small and J. J. Carter in Dade County. Small (1931, 1938) knew of this species only from Ross Hammock near Silver Palm. Searches by myself and others as recently as 1981 have failed to locate plants at this site. There has been little disturbance at Ross Hammock that would explain its apparent absence. To my knowledge, no one has yet searched for gametophytes.

Trichomanes petersii A. Gray—This species can be distinguished from the other simple-leaved taxa by a midrib that extends to the leaf apex, a fully immersed involucre, and flaring, undulate involucre lips that are otherwise little distinct from the lamina tissue.

Distribution and habitat.—Citrus, Hernando, and Sumter counties, Florida, northward in the Southeastern United States from Georgia, Alabama, and Louisiana north to North Carolina and Tennessee, and south to Mexico and Guatemala; epipetric on sandstone or other acidic rocks or epiphytic in moist habitats, often near water. Sporulates in summer.

Trichomanes petersii was first collected in Florida in 1936 by E. St. John in Hernando County. The species appears to be restricted to acid substrates in spite of reports to the contrary (Brass, 1955; Duncan, 1955). Wessels Boer (1962) reported it as epiphytic or epipetric on sandstone and limestone rocks. He apparently based his inclusion of limestone as a substrate on herbarium material, including a collection by E. St. John from Brooksville. Wherry (1955) reported that a visit to this site revealed plants occurring on only two boulders, both of which were chert, not limestone. This strengthened Wherry's contention that the species occurs only on acid substrates.

The occurrence of the species as an epiphyte seems to be more prevalent in southern portions of its range, and occurrence on rocks is more common in northern portions of its range. In relatively warmer regions the species is known largely as an epiphyte (Stolze, 1976; Wessels Boer, 1962). The American Institute of Biological Sciences field trips in 1986 revealed plants in Sumter County occurring as epiphytes on oaks in a swamp system near the Withlacoochee State Forest. Farther north, *T. petersii* occurs on wet, acid rock substrates in protected crevices, usually near running water (e.g., Shaver, 1954, and references therein; Wyatt, 1984). Lower temperature regimes may be critical in determining whether it grows epiphytically or epipetrically.

Trichomanes punctatum Poiret in Lam. subsp. **floridanum** W. Boer

Distribution and habitat.—Citrus, Sumter, and Dade counties, Florida; endemic; epiphytic or epipetric on limestone in moist forested habitats. Other subspecies

occur throughout the American tropics. Lellinger (1985) reported only Dade and Sumter counties as the extant range for this species. Sporulates all year, but mostly in spring and summer northward.

This subspecies was first collected in Florida in 1901 by J. K. Small in Dade County. Plants were first collected in Citrus and Sumter counties in 1936 by R. St. John. Small (1931, 1938) listed Snapper Creek Hammock, and several pineland hammocks as far southwest as Camp Longview and Royal Palm Hammock. Additionally, the plant has been collected south of Cutler, and at Hattie Bauer, Colwell, Sykes, and Ross hammocks by A. A. Eaton, J. K. Small, and others. The plants are still known to occur in Fuchs and Castello hammocks in Dade County and are apparently absent at the other stations.

Small (1918a, 1918b, 1931) used the name *T. punctatum*, but in 1938 he misapplied the name *T. sphenoides* Kunze to the Citrus and Sumter County populations. The misapplication was followed by Wherry (1964). Both authors were apparently convinced that the disjunction of the two populations was partly due to their being different taxa, or at least was sufficient to judge them to be different taxa. Yet, these same authors were willing to accept the disjunct populations of *T. petersii* in Florida. Perhaps their greater familiarity with populations of *T. petersii* in more northern states allowed them greater confidence in applying the name to the disjunct Florida populations. A lack of familiarity with *T. sphenoides*, its reported wide distribution in the American tropics (especially in the Antilles), and Eaton's (1906) misapplication of the name to Dade County populations, made it a logical possibility for the northern stations of *T. punctatum* subsp. *floridanum*.

Conservation Status

All five taxa have at least one population represented within a Federal, State, or local park or preserve. This does not however, guarantee their preservation. The taxa inhabiting swamps, *T. holopterum* and *T. petersii*, may undergo some recovery if habitats are not modified in those areas under protection. Taxa occurring in hammocks on limestone, *T. krausii*, *T. punctatum* ssp. *floridanum*, and *T. lineolatum*, are in danger of extirpation, despite protective efforts. These species appear to require high relative atmospheric humidities. Sinkholes with year-round standing water and dense canopies once maintained such high relative humidities in the hammock habitats. Drainage efforts in Florida since the early 1900s, however, have sufficiently lowered the water table that the sinkholes no longer contain standing water for any significant period of time. Additionally, human disturbance has opened many hammocks to the desiccating effects of wind and sun. As a result, one species (*T. lineolatum*) may have already been lost, and two others are in immediate danger of extirpation.

Excluded Species

Trichomanes boschianum Sturm—This plant was listed by Small (1931, 1938) as questionably occurring in western Florida, stating in 1938 (p. 57), "The Flor-

ida station for it appears to be lost to science." The species has been excluded from Florida by all subsequent authors. Since I know of no documented records in Florida, I am excluding it.

Trichomanes sphenoides Kunze (≡*T. punctatum* ssp. *sphenoides* (Kunze) W. Boer)—Misapplied to *T. punctatum* ssp. *floridanum* by Eaton (1906), Small (1938), and Wherry (1964). This subspecies occurs in the Greater Antilles, Central, and northwestern South America (Wessels Boer, 1962).

Trichomanes punctatum ssp. *sphenoides* (Kunze) W. Boer—Treated as a synonym of ssp. *floridanum* by Lakela and Long (1976).

Trichomanes membranaceum L.—Mistakenly cited for Florida by Mickel (1979). Mickel has confirmed this (pers. comm.).

Acknowledgments

I thank Dr. R. P. Wunderlin, Univ. of South Florida, Tampa, A. M. Evans, Univ. of Tennessee, Knoxville, and reviewers for their comments and critique.

Literature Cited

Brass, L. J. 1955. Report of the southern Florida field trip. Amer. Fern J. 45:48–54.

Delchamps, C. A. 1966. *Trichomanes holopterum*—a filmy fern new to the United States. Amer. Fern J. 56:138–139.

Duncan, W. H. 1955. New records for Georgia ferns. Amer. Fern J. 45:1–10.

Eaton, A. A. 1906. Pteridophytes observed during three excursions into southern Florida. Bull. Torrey Bot. Club 33:455–486.

Farrar, D. R. and W. H. Wagner, Jr. 1968. The gametophytes of *Trichomanes holopterum* Kunze. Bot. Gaz. (Crawfordsville) 129:210–219.

Lakela, O. and R. W. Long. 1976. *Ferns of Florida*. Miami: Banyan Books.

Lellinger, D. B. 1985. *A field guide of the ferns & fern allies of the United States & Canada*. Washington, DC: Smithsonian Institution Press.

Mickel, J. T. 1979. *How to know the ferns and fern allies*. Dubuque, Iowa: W. C. Brown Co.

Morton, C. V. 1968. The genera, subgenera, and sections of the Hymenophyllaceae. Contr. U. S. Natl. Herb. 38:153–214.

Proctor, G. R. 1977. Pteridophyta. Vol. 2 of *Flora of the Lesser Antilles*, ed. R. A. Howard. Jamaica Plain, Mass.: Arnold Arboretum.

———. 1985. *Ferns of Jamaica*. London: British Museum (Nat. Hist.).

Shaver, J. M. 1954. *Ferns of the eastern central states with special reference to Tennessee*. New York: Dover Publ.

Small, J. K. 1918a. *Ferns of Royal Palm Hammock*. New York: Publ. by the author.

———. 1918b. *Ferns of Tropical Florida*. New York: Publ. by the author.

———. 1931. *Ferns of Florida*. New York: Science Press.

———. 1938. *Ferns of the Southeastern States*. Lancaster, Penn: Science Press.

Stolze, R. G. 1976. Ferns and fern allies of Guatemala, Part I. Ophioglossaceae through Cyatheaceae. Fieldiana, Bot. 39:1–130.

Wessels Boer, J. G. 1962. The new world species of *Trichomanes* sect. *Didymoglossum* and *Microgonium*. Acta Bot. Neerl. 11:277–330.

Wherry, E. T. 1955. The substratum of *Trichomanes petersii*. Amer. Fern J. 45:93–95.

———. 1964. *The southern fern guide*. Garden City, New York: Doubleday & Co.

Wyatt, R. 1984. A new station for *Trichomanes petersii* in Alabama. Amer. Fern J. 74:30.

American Fern Journal 76(4):184–186 (1986)

Some New Names and Combinations in Pteridaceae

ROLLA TRYON
Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138

Although Pteridaceae Reichb. f. has been considered to be an illegitimate name (Pichi Sermolli, 1982), Article 63.3 of the International Code of Botanical Nomenclature was altered at Sydney and the name is consequently legitimate. It may, however, be antedated by Pteridaceae Gaud. or Pteridaceae (S. F. Gray) Gaud. and for this reason Panigrahi (1986) has proposed the name for conservation with appropriate authorship. The name was used by Tryon and Tryon (1982) and it will be used in "The Families and Genera of Vascular Plants" in anticipation of its conservation. Adopted in a broad sense, the Pteridaceae include major groups of ferns that are currently recognized, sometimes as tribes and sometimes as families. In order to be consistent with other treatments of fern families in "The Families and Genera of Vascular Plants," it is necessary to place them as subfamilies. The nomenclature of these follows with the new status provided when necessary.

Pteridaceae subfam. **Adiantoideae** (Presl) R. Tryon, stat. nov.—Adianteae, as tribe Adiantaceae Presl, Tent. Pterid. 139. 1836.

Pteridaceae subfam. **Ceratopteridoideae** (J. Smith) R. Tryon, stat. nov.—tribe Ceratopterideae J. Smith, Hist. Fil. 170. 1875.

Pteridaceae subfam. **Cheilanthoideae** (J. Smith) R. Tryon, stat. nov.—tribe Cheilantheae J. Smith, Hist. Fil. 277. 1875.

Pteridaceae subfam. Platyzomatoideae (Nakai) A. F. Tryon, Amer. J. Bot. 51:942. 1964.

Pteridaceae subfam. Pteridoideae.

Pteridaceae subfam. **Taenitidoideae** (Presl) R. Tryon, stat. nov.—tribe Taenitideae Presl, Tent. Pterid. 222. 1836

Sections of Syngramma.—Three sections are recognized in the genus: sect. *Syngramma* and sect. *Toxopteris*, both with a simple lamina, and sect. *Craspedodictyum* with a ternate, pedate, or palmate lamina.

Well developed leaves of the six species of sect. *Craspedodictyum* have a 3-foliolate to 5-(rarely 6-)foliolate lamina and represent a distinct group within the genus. In a study of *Syngramma* and related genera, Holttum (1975) recognized that *Craspedodictyum* did not merit generic rank and he placed the species in sect. *Syngramma* in a group "with pedate fronds."

Syngramma sect. **Craspedodictyum** (Copel.) R. Tryon, stat. nov.—*Craspedodictyum* Copel., Philipp. J. Sci., C. 6:84. 1911.

Species of Cerosora.—A third species is added to the genus *Cerosora*, along with *C. chrysosora* (Baker) Domin of Borneo and the recently described *C. sumatrana* Holtt. of Sumatra. This is the previously misunderstood *Gymnogramma microphylla* or *Pityrogramma microphylla* (Hook.) Domin of the Himalayas.

The characters of the two previous species provide a clear separation of *Cerosora* from other genera of the Pteridaceae: stem creeping, with rigid, dark trichomes or bristles, petiole with one vascular bundle near the base, veins all free, exindusiate sori along the veins with few-celled, simple trichomes among the sporangia, and spores with a prominent equatorial flange. The species *C. microphylla* has these characters and is clearly a member of the genus.

6050

Cerosora microphylla (Hook.) R. Tryon, comb. nov.—*Gymnogramma microphylla* Hook., Icon. Pl. 10:t. 916 (Cent. Ferns t. 16). 1854.

Cheilanthes smithii—The species *Pellaea smithii*, with unusual long and narrow fertile segments and corresponding long, modified marginal indusia, is placed in *Cheilanthes*. It was included, along with related species of eastern Asia, in *Mildella*, a segregate genus from *Cheilanthes* by Hall and Lellinger (1967). As in *Cheilanthes* species, the lamina of *C. smithii* is more or less gradually reduced apically and the ultimate segments are mostly joined or a few are adnate. *Pellaea* species differ in their imparipinnate lamina and stalked to sessile ultimate segments.

6051

Cheilanthes smithii (C. Chr.) R. Tryon, comb. nov.—*Pellaea smithii* C. Chr., Acta Horti Gothob. 1:84. 1924.

The genus Paraceterach.—*Paraceterach* is enlarged to include five Asiatic species often treated in *Gymnopteris* or *Hemionitis*, along with the two Australian species previously included in the genus. It is characterized by the following: lamina 1- or 2-pinnate, on the lower surface densely invested with indument which conceals the sporangia (especially the young ones), exindusiate soral lines on unmodified, free, or occasionally anastomosing, veins, and spores that are cristate, echinate or plain (the surface deposit incomplete). This suite of characters serves to distinguish the genus from related ones such as *Coniogramme*, *Bommeria*, and *Hemionitis*.

Three groups may be recognized in the genus. The Australian species, *P. muelleri* and *P. reynoldsii*, have a moderately creeping stem bearing mature scales with a dark, sclerotic center and lighter margins, and subclathrate, concolorous lamina scales. An Asiatic group of two species, *P. delavayi* and *P. marantae*, (the latter also westward to Europe) has short-creeping, multicipital stems, with light brown to somewhat reddish, narrow, concolorous scales, and the lamina indument is similar to that in the Australian species. The other Asiatic group, composed of *P. bipinnata*, *P. sargentii*, and *P. vestita*, differs only in having the lamina indument of very long, appressed and matted trichomes. These groups represent three lineages that can be accommodated within *Paraceterach* as are distinctive elements within *Cheilanthes*, *Notholaena*, *Pellaea*, and *Hemionitis*.

Hemionitis is an American genus of seven species, differing from *Paraceterach* especially in the indument on the lower surface of the segments. There are few or no scales, and the few to numerous trichomes are short (longer ones are also present in two species with dimorphic pubescence), acicular, and erect or patent, and the soral lines of sporangia are evident. In addition, some species

have an erect stem, and most of them have an entire to deeply lobed lamina. *Hemionitis* and *Paraceterach* evidently represent separate, convergent lineages. Isozyme studies will be especially useful for depicting relations of these genera.

"*Hemionitis*" *arifolia* is reported by Manton & Sledge (1954) as an apogamous tetraploid species. The chromosome number of n and $2n = 120$ clearly relates it to the Pteridaceae and especially to the subfam. Cheilanthoideae. It is a morphologically isolated species of uncertain affinity and likely represents a derived alloploid. Giannasi and Mickel (1979) identified seven flavonoids common to *Hemionitis arifolia* and *H. elegans*, and also reported four flavonoids unique to *H. arifolia*.

It is recognized that *Acrostichum marantae* L., here included in *Paraceterach*, is sometimes considered to be the type of *Notholaena* (Pichi Sermolli, 1983, pp. 112–117). However, John Smith (1875, p. 278) was the first to typify *Notholaena* and his choice, *Pteris trichomanoides* L. [*Notholaena trichomanoides* (L.) Desv.] cannot be superseded.

The species of *Paraceterach* are the following:

Paraceterach bipinnata (Christ) R. Tryon, comb. nov.—*Gymnopteris bipinnata* Christ, Notul. Syst. (Paris) 1:55. 1909.

Paraceterach delavayi (Baker) R. Tryon, comb. nov.—*Gymnogramma delavayi* Baker, Ann. Bot. (London) 5:484. 1891.—*Gymnopteris delavayi* (Baker) Underw.

Paraceterach marantae (L.) R. Tryon, comb. nov.—*Acrostichum marantae* L., Sp. Pl. 1071. 1753.—*Gymnopteris marantae* (L.) Ching.

Paraceterach muelleri (Hook.) Copel., Gen. Fil. 75. 1947.

Paraceterach reynoldsii (F. Muell.) Tindale, in J. M. Black, Fl. South Australia, ed. 3, by J. P. Jessop, 1:53. 1978.

Paraceterach sargentii (Christ) R. Tryon, comb. nov.—*Gymnopteris sargentii* Christ, Bot. Gaz. (Crawfordsville) 51:355. 1911.

Paraceterach vestita (Hook.) R. Tryon, comb. nov.—*Gymnogramma vestita* Hook., Icon. Pl. t. 115. 1837.—*Gymnopteris vestita* (Hook.) Underw.

Literature Cited

Giannasi, D. E. and J. T. Mickel. 1979. Systematic implications of flavonoid pigments in the fern genus *Hemionitis* (Adiantaceae). Brittonia 31:405–412.

Hall, C. C. and D. B. Lellinger. 1967. A revision of the fern genus *Mildella*. Amer. Fern J. 57: 113–134.

Holttum, R. E. 1975. A comparative account of the fern-genera *Syngramma* J. Sm. and *Taenitis* Willd. Kew Bull. 30:327–343.

Manton, I. and W. A. Sledge. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Philos. Trans. Ser. B. 238:127–185.

Panigrahi, G. 1986. Proposal to conserve Pteridaceae Reichenbach (Pteridophyta). Taxon 35:385–386.

Pichi Sermolli, R. E. G. 1982. A further contribution to the nomenclature of the families of Pteridophyta. Webbia 35:223–337.

———. 1983. Fragmenta Pteridologiae, VIII. Webbia 37:111–140.

Smith, J. 1875. *Historia filicum*. London: Macmillan & Co.

Tryon, R. M. and A. F. Tryon. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.

American Fern Journal 76(4):187–188 (1986)

A Novel Method for Surface-sterilizing and Sowing Fern Spores

THOMAS R. WARNE, GARY L. WALKER, and LESLIE G. HICKOK
Department of Botany, University of Tennessee, Knoxville, Tennessee 37996-1100

Numerous methods are available for the sterilization of fern spores (see Dyer, 1979). Many of these methods are inefficient in terms of time and spore loss and require bulky equipment such as a centrifuge or vacuum filtration units. We present an easy and effective method for spore sterilization that requires no specialized tools or equipment, other than a sterile area. We have used the method presented here routinely to sterilize *Ceratopteris richardii* Brongn. spores. In addition, this technique has worked with spores of *Lygodium*, *Pteridium*, and other species of *Ceratopteris* (unpublished data).

The basic innovation in this method involves the removal of liquid from a spore suspension in a conical centrifuge tube by means of a Pasteur pipet while leaving the spores in place. This method requires the following supplies: 1) sterile Pasteur pipets (Fisherbrand® borosilicate glass, 5¾ inches) with square-cut ends that are not cracked or chipped; 2) sterile pipet bulbs (natural rubber dropper bulb); and 3) sterile conical centrifuge tubes (Pyrex® No. 8060, 15 ml). To accomplish spore removal, insert a pipet with attached bulb into a conical centrifuge tube that contains a liquid and spores and suspend the spores in solution by bubbling air into the liquid. While continuing to bubble air, gently, but securely, seat the pipet tip onto the base of centrifuge tube. A slight rotation of the pipet may assist seating, but excess downward or lateral force will crush the pipet tip. Squeeze the pipet bulb to force additional air out of pipet, then slowly release the bulb to aspirate liquid into the pipet; most spores should collect at the base of the centrifuge tube and not be drawn into the pipet. This procedure has been incorporated into the following generalized spore sterilization and sowing method.

Spore preparation.—These procedures can be performed outside of a sterile area. Transfer spores into a conical centrifuge tube and presoak them with distilled water for 16 hours. We routinely dry-sterilize (120°C, 2 hours) centrifuge tubes to eliminate cross-contamination of spore stocks and minimize contamination from the tube. If the spore source is exceptionally dirty or contaminated, wetted spores may be transferred with a Pasteur pipet to a clean sterile centrifuge tube immediately prior to sterilization. This significantly reduces the amount of coarse debris that may harbor and shield contaminants during sterilization. We prepare and sterilize from 2 to 250 mg of *Ceratopteris* spores per tube, depending on the desired sowing density and number of cultures to be sown.

Spore sterilization.—All procedures for spore sterilization and spore sowing should be performed in a sterile area using standard sterile technique. All materials that come into contact with spores should be sterilized. To avoid contamination, use a clean sterile pipet and pipet bulb for each step.

To sterilize spores, remove the presoak solution using the method described

above and discard the pipet used. Add ca. 2.0 ml or one full Pasteur pipet of freshly prepared 0.875% sodium hypochlorite (1 part commercial bleach, 5.25% sodium hypochlorite, to 5 parts distilled water). To insure adequate sterilization of spores and centrifuge tube interior, add this solution so that it washes from the lip of the centrifuge tube and suspend the spores in this solution by bubbling air through a clean, sterile pipet.

Remove the sodium hypochlorite solution with a clean, sterile pipet using the technique described above. Immediately rinse the spores three times. For each rinse, add one full pipet of sterile distilled water to the tube and remove water with a clean, sterile pipet using the above method. For sterilization, we routinely expose *Ceratopteris* spores to the sodium hypochlorite solution for 2½ minutes.

Spore sowing.—For spore sowing, add a known amount of sterile distilled water to the sterilized spores and uniformly suspend spores by bubbling air in solution. Dispense drops of the spore suspension into the appropriate culture vessel containing liquid or agar-solidified medium, soil, etc. For agar-solidified medium, spores can be uniformly distributed across the agar surface with a sterile wire bent into a smooth cornered triangle (coat hanger shape).

We routinely use 5 Pasteur pipet drops of spore suspension (ca. 0.146 ml) per culture vessel. Controlled-drop pipets may make sowing more precise. For controlled sowings it is necessary to predetermine the number of spores per unit weight and to correct for maximum percentage germination of a given spore stock. For example, we have determined that in 1.0 mg dry weight there are ca. 1250 *C. richardii* spores and that maximum percentage germination may range from 60 to 95% for different sources.

Modifications for spores of other taxa.—Adjustments that may be needed in presoak and sterilization times and solutions, depending upon the spores used, can be empirically determined. Sterilization of spores smaller than those of *Ceratopteris*, which are ca. 86–129 μm diameter (Lloyd, 1974), may require careful selection of both Pasteur pipets and centrifuge tubes and careful practiced technique.

ACKNOWLEDGMENTS

This research was supported by grant DCB-85-11273 from the National Science Foundation to L. Hickok and T. Warne.

LITERATURE CITED

DYER, A. F. 1979. The culture of fern gametophytes for experimental investigation. Pp. 253–305 in *The experimental biology of ferns*, ed. A. F. Dyer. London: Academic Press.

LLOYD, R. M. 1974. Systematics of the genus *Ceratopteris* Brongn. (Parkeriaceae) II. Taxonomy. Brittonia 26:139–160.

Shorter Note

***Osmunda cinnamomea* forma *frondosa* in the Coastal Plain of Georgia and Florida.**—The article of Werth et al. (Amer. Fern J. 75:128–132, 1985) reporting the discovery and observations of *Osmunda cinnamomea* L. forma *frondosa* (Torrey & Gray) Britton at Mountain Lake, Virginia, has provoked interest in this puzzling morphology. Typically, *O. cinnamomea* sporophylls are strictly dimorphic with respect to spore production; however, forma *frondosa* produces sporophylls that bear both sterile and fertile pinnae. This unusual form of Cinnamon Fern has not been reported previously from Georgia (McVaugh & Pyron, *Ferns of Georgia*, The University of Georgia Press, 1951) or Florida (Lakela & Long, *Ferns of Florida*, Banyan Books, Miami, 1976). However, there are at VSC three records of *Osmunda cinnamomea* forma *frondosa* from the Coastal Plain of Georgia and Florida:

Georgia: **Brantley Co.:** ¼ mi E of ITT-Rayonier Satilla Forest Headquarters along Hwy GA-32 between Browntown and Needmore, 9 Sept 1982, *Faircloth 8711*. **Lowndes Co.:** Slough S of Valdosta, along Loch Laurel Road, 4.3 mi S of Hwy GA-31, 24 Apr 1986, *Carter 4807*. Florida: **Leon Co.:** Disturbed sandy ground just E of St. Marks River and 2 mi W of Jefferson County line on Hwy US-27, 1 Nov 1979, *Carter 2318*.

The Brantley County population consisted of seven plants that were located on the backslope of highway GA-32 and numerous plants that extended into the adjacent pine flatwoods. Only those plants located on the backslope had *frondosa*-type sporophylls. Development of laminar and sporogenous pinnae on a frond was highly variable among the seven plants and even among fronds on individual plants. There was evidence that the plants on the backslope had been mown over earlier in the year by Rayonier personnel. At the Lowndes County site only a single plant of forma *frondosa* was found in a population of about one hundred typical plants. This plant was located near the edge of woods and was conspicuously smaller than its typical neighbors. Data on population size and other pertinent notes relating to site disturbance is lacking for the Leon County record. Our observations also suggest that this phenomenon is environmentally induced but we have no additional explanations to offer beyond those reviewed and proposed by Werth et al. (1985). The phenomenon probably has little genetic or taxonomic significance; however, we feel it is worthy of continued study to elucidate a clearer explanation of the factors underlying morphological expression of leaf dimorphism. We do suspect that forma *frondosa* is of more frequent and widespread occurrence than has been reported previously.—Richard Carter and Wayne R. Faircloth, Department of Biology, Valdosta State College, Valdosta, GA 31698.

Review

"*Polypodiaceous Ferns of India*," by C. K. Satija and S. S. Bir. 1985. vi + 132 pp. including 9 photographic plates. Aspects of Plant Sciences, Volume 8. New Delhi: Today & Tomorrow's Printers and Publishers. Distributed in U.S.A. and Canada by: Scholarly Publications, 7310 El Cresta Drive, Houston, TX 77083. $19.00.

This is a listing of Indian Polypodiaceae in the strict sense, with also the Grammitids, *Loxogramme*, and *Dipteris*. For each species there are selected references, ecological comments, and a statement of distribution, but no keys or descriptions. Following the main text are two addenda and an appendix by S. S. Bir, a very incomplete three pages of errata, and an index.

Some species that were included solely on the basis of literature reports are apparent misidentifications or mislocalizations, whereas others that I believe to be reliably on record from India are unmentioned. I also noted five species originally described from India that were omitted: *Grammitis cuspidata* Zenker, Plantae Indicae 1, t.2. 1835 (a *Loxogramme*); *Grammitis attenuata* Kunze, Linnaea 24:251. 1851 (a true *Grammitis*); *Polypodium decurrenti-adnatum* Rosenstock, Repert. Spec. Nov. Regni Veg. 12:248. 1913; *Drynaria meeboldii* Rosenstock, ibid.; and *Arthromeris jarrettiae* Sastry & Chowdhury, Bull. Bot. Surv. India 11:442. 1972.

Four combinations are proposed in *Phymatopteris* but two of these were already published by Pichi Sermolli in Webbia 28 (1973) in a paper cited several times elsewhere in the book. As a genus, *Phymatopteris* Pic. Ser. can hardly stand as it merges with the earlier *Crypsinus* Presl.—M. G. Price, Herbarium, North University Building, University of Michigan, Ann Arbor, MI 48109.

Referees, 1986

I thank the Associate Editors and referees listed below for their valuable assistance in the review process. Their evaluations of manuscripts submitted to American Fern Journal have aided authors, made my job easier, and contributed to the quality of our journal.—Alan R. Smith

Donald M. Britton
Howard Calkin
David S. Conant
Gillian Cooper-Driver
Michael Cousens
Barbara Ertter
A. Murray Evans
Donald R. Farrar
Arthur C. Gibson
Judith E. Gordon
Leslie D. Gottlieb
R. James Hickey
David M. Johnson
Robert M. Lloyd
Michael R. Mesler
John T. Mickel
James D. Montgomery
Robbin C. Moran
Suzanne Morse
Michael G. Price
George R. Proctor
Thomas Ranker
Douglas E. Soltis
Dennis Wm. Stevenson
W. Carl Taylor
David H. Wagner
Warren H. Wagner, Jr.
James W. Wallace
Charles R. Werth
Dean P. Whittier
Michael D. Windham
George Yatskievych

American Fern Journal 76(4):191 (1986)

Index to Volume 76—1986

Classified entries: botanical names (new names in **boldface**); major subject headings, including key words from titles; reviews (grouped, listed by first author of work reviewed). Names of authors, followed by titles of articles or references to first authors, are listed alphabetically in Table of Contents, iii–iv.

Adiantum pedatum complex, 151
Anatomy: Cyclodium, 57; Olfersia, 163
Asplenium septentrionale, 26
Botrychium, 33: in western North America, 33; subg. Botrychium, 33; **B. ascendens,** 36; B. ascendens × crenulatum, 39; **B. campestre,** 39; **B. pedunculosum,** 43; B. pedunculosum × pinnatum, 45
Cerosora, 184; **C. microphylla,** 185
Cheilanthes smithii, 185
Chromosome number: Botrychium ascendens, 38; B. campestre, 42; B. pedunculosum, 43; Cyclodium meniscioides, 64; Pellaea breweri, 118; Pellaea truncata, 119
Conservation: Trichomanes in Florida, 182
Corrections, Index filicum, 47
Cultural techniques: Isoëtes spp., 21; Lycopodium lucidulum, 48
Cyclodium, 56: **C. akawaiorum,** 71; **C. calophyllum,** 73; **C. guianense,** 75; C. heterodon var. heterodon, 79; **C. heterodon** var. **abbreviatum,** 80; **C. inerme,** 82; C. meniscioides var. meniscioides, 87; **C. meniscioides** var. **paludosum,** 87; **C. meniscioides** var. **rigidissimum,** 88; **C. rheophilum,** 88; **C. seemannii,** 89; **C. trianae** var. **trianae,** 92; **C. trianae** var. **chocoense,** 93; **C. varians,** 94
Cystopteris tennesseensis, 99
Dryopteris carthusiana, 28
Ecology, Cyclodium, 64
Electrophoresis: in pteridophytes, 102; Marsilea, 17; Regnellidium, 17
Endemism: Botrychium, 45
Enzymes: in herbarium specimens, 102; Marsilea, 17; Regnellidium, 17
Gametophytes: biosystematic uses of, 114; Isoëtes spp., 21; Lycopodium lucidulum, 48
Geographic variation: in Pellaea, 120
Herbarium specimens: in biosystematic research, 101; enzymes from, 102; gametophytes from, 114; spore abortion in, 129; spore size in, 149; spore viability in, 141; stomate size in, 149
Hemionitis, 185; H. arifolia, 186
Hybridization: Botrychium, 39, 45; Cyclodium, 64; Pellaea, 121
Index filicum, corrections, 47
Isoëtes: megaspore morphology, 1; spore terminology, 1
Lycopodium lucidulum, 48
Marsilea vestita, 17
Megaspores: Isoëtes, 1; Marsilea, 17; Regnellidium, 17
Morphology: Cyclodium, 57; Olfersia, 163
Olfersia, 161; O. cervina, 161
Ophioglossum palmatum, 55
Osmunda cinnamomea f. frondosa, 189
Paraceterach, 185: **P. bipinnata,** 186; **P. delavayi,** 186; **P. marantae,** 186; P. muelleri, 186; P. reynoldsii, 186; **P. sargentii,** 186; **P. vestita,** 186
Pellaea: P. atropurpurea, 142; P. brachyptera, 25; P. breweri, 118; P. ternifolia, 142; P. truncata, 119; P. wrightiana, 142
Polybotrya, 65, 161
Polypodium: spore size, 155; stomate size, 155; P. californicum, 155; P. glycyrrhiza, 155
Polystichum: spore size, 153; P. acrostichoides, 157; P. braunii, 157; P. polyphyllum, 153; P. × potteri, 158
Pteridaceae, 184: subfam. **Adiantoideae,** 184; subfam. **Ceratopteridoideae,** 184; subfam. **Cheilanthoideae,** 184; subfam. Platyzomatoideae, 184; subfam. Pteridoideae, 184; subfam. **Taenitidoideae,** 184
Range extensions: Arkansas, Dryopteris carthusiana, 28; Georgia and Florida, Osmunda cinnamomea f. frondosa, 189; Washington, Pellaea brachyptera, 25; West Virginia, Asplenium septentrionale, 26; West Virginia, Cystopteris tennesseensis, 99
Referees, 1986, 190
Regnellidium diphyllum, 17
Reviews: Dyer, A. F. and C. N. Page, eds., Biology of pteridophytes, 30; Lellinger, D. B., A field manual of the ferns & fern-allies of the United States & Canada, 29; Satija, C. K. and S. S. Bir, Polypodiaceous ferns of India, 190
Spores: abortion, 129; size in Adiantum pedatum complex, 152; viability in Pellaea, 141; Cyclodium, 63; Isoëtes, 1, 21; Olfersia, 161
Spore germination: Isoëtes, 21
Spore sterilization and culture, 187
Stigmatopteris, 56
Syngramma, 184: sect. **Craspedodictyum,** 184; sect. Syngramma, 184; sect. Toxopteris, 184
Trichomanes: in Florida, 179; T. holopterum, 179; T. krausii, 180; T. lineolatum, 180; T. petersii, 181; T. punctatum subsp. floridanum, 181

U.S. Postal Service

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

Required by 39 U.S.C. 3685)

1A. TITLE OF PUBLICATION	1B. PUBLICATION NO.	2. DATE OF FILING
AMERICAN FERN JOURNAL	0 0 0 2 8 4 4 4	18 Nov 1986

3. FREQUENCY OF ISSUE	3A. NO. OF ISSUES PUBLISHED ANNUALLY	3B. ANNUAL SUBSCRIPTION PRICE
quarterly	4	$10 mem $12 subscr

4. COMPLETE MAILING ADDRESS OF KNOWN OFFICE OF PUBLICATION *(Street, City, County, State and ZIP Code) (Not printers)*

Pringle Herbarium, University of Vermont, Burlington, VT 05405

5. COMPLETE MAILING ADDRESS OF THE HEADQUARTERS OF GENERAL BUSINESS OFFICES OF THE PUBLISHER *(Not printer)*

Pringle Herbarium, University of Vermont, Burlington, VT 05405

6. FULL NAMES AND COMPLETE MAILING ADDRESS OF PUBLISHER, EDITOR, AND MANAGING EDITOR *(This item MUST NOT be blank)*

PUBLISHER *(Name and Complete Mailing Address)*

AMERICAN FERN SOCIETY, INC. (see 5.)

EDITOR *(Name and Complete Mailing Address)*

Dr. Alan R. Smith, Dept. of Botany, Univ. of California, Berkeley, CA 94720

MANAGING EDITOR *(Name and Complete Mailing Address)*

none

7. OWNER *(If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Item must be completed.)*

FULL NAME	COMPLETE MAILING ADDRESS
American Fern Society, Inc.	(see 5.)

8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES *(If there are none, so state)*

FULL NAME	COMPLETE MAILING ADDRESS
none	

9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES *(Section 423.12 DMM only)*
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes *(Check one)*

[X] (1) HAS NOT CHANGED DURING PRECEDING 12 MONTHS

[ ] (2) HAS CHANGED DURING PRECEDING 12 MONTHS *(If changed, publisher must submit explanation of change with this statement.)*

10. EXTENT AND NATURE OF CIRCULATION	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES *(Net Press Run)*	1350	1400
B. PAID CIRCULATION 1. Sales through dealers and carriers, street vendors and counter sales	0	0
2. Mail Subscription	1214	1222
C. TOTAL PAID CIRCULATION *(Sum of 10B1 and 10B2)*	1214	1222
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	0	0
E. TOTAL DISTRIBUTION *(Sum of C and D)*	1214	1222
F. COPIES NOT DISTRIBUTED 1. Office use, left over, unaccounted, spoiled after printing	136	178
2. Return from News Agents	0	0
G. TOTAL *(Sum of E, F1 and 2–should equal net press run shown in A)*	1350	1400

11. I certify that the statements made by me above are correct and complete

SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER

Alan R. Smith

Alan R. Smith, editor

PS Form 3526 July 1982

(See instruction on reverse)

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations. Do not send originals of illustrations until they are requested. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Avoid footnotes and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by a uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matters of form or style, consult recent issues of *American Fern Journal* and *The Chicago manual of style*, 13th ed. (1982. Chicago: Univ. Chicago Press). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).